Survey for the presence of Mycotoxins with special reference to Patulin, Sterigmatocystin, Ochratoxin and Penicillic Acid in Foods, Food Products, Animal Feeds and Concentrates available in the Tamil Nadu Region of India

1976 - 1979

FINAL REPORT

DEPARTMENT OF FOOD TECHNOLOGY
TAMIL NADU AGRICULTURAL UNIVERSITY
COIMBATORE - 641 003
INDIA

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Tamil Nadu Agricultural University

A. VENKATARAMAN, I.A.S.,
VICE-CHANCELLOR

COIMBATORE - 641 003

4th March 1980

FOREWORD

Malnutrition is one of the maladies from which our people and livestock suffer from. This already bad situation is many times made more worse by making available contaminated food and feed to them due to poor handling of the food-grains and inadequate knowledge or ignorance on mycotoxins. All food and food products are susceptible for mould attack. High temperature coupled with high humidity normally present for long periods in vast areas of our country favour rapid mould growth and presence of aflatoxins in food and feed grains and processed products. Sustained efforts should be taken to monitor for harmful toxins in these materials and make them unfit for any consumption. Besides information on the harmful effects of these toxins and methods to avoid them must be continuously popularised among the public.

Results of a research project on survey for the presence of mycotoxins in foods, food products, animal feeds and concentrates available in the Tamil Nadu Region of India conducted by the Food Technology Department of the Tamil Nadu Agricultural University are presented in this book. I wish to compliment the scientists for their contribution and record my thanks to Government of India for having provided us with the necessary funds for conducting this project. I am confident that the findings will be put to use by the authorities concerned with "food and nutrition" development and "Health Care".

(Signed)

(A. Venkataraman)

ACKNOWLEDGEMENTS

We are grateful to the Government of India for the generous financial grant for carrying out this survey and to Thiru. A. Venkataraman, I. A. S., Vice Chancellor of this University for his keen interest and for having written the foreword for this Final Report. Our thanks are due also to the following who have helped in many ways.

1. Dr. Glenn Bennett, Chemist, Mycotoxin Analytical and Chemical Research, Fermentation Laboratory, NRRL, Peoria, Illinois, U.S.A.

2. Dr. Nathan L. Brown, Department of Health, Education and Welfare (HEW), Washington D.C., U.S.A.

3. Dr. L. B. Bullerman, University of Nebraska, Lincoln, Nebraska, U.S.A.

4. Dr. Alex Ciegler, NRRL, ARS-U.S.D.A., Peoria, Illinois, U.S.A.

5. Dr. Richard Cole, Research Microbiologist, National Peanut Research Laboratory, Dawson, Georgia, U.S.A.

6. Dr. L. L. Flag, Dept. of Plant Pathology, UPLB College of Agriculture, Phillipines.

7. Dr. C. W. Hesseltine, Chief, Fermentation Laboratory, Northern Regional Research Laboratory, Peoria, Illinois, U.S.A.

8. Dr. Joseph Lovet, Division of Microbiology, FDA, Washington D.C., U.S.A.

9. Dr. Meridith, U.S.D.A., A.R.S., Northern Regional Research Laboratory, Peoria, Illinois, U.S.A.

10. Dr. C. T. Mirocha, Laboratory of Mycotoxicology, University of Minnesota, St. Paul, Minnesota, U.S.A.

11. Dr. A. Schindler, Research Plant Pathologist, Dept. of HEW, FDA, Washington D.C., U.S.A.

12. Dr. H. W. Schroeder, Research Leader, U.S.D.A., College Station, Texas, U.S.A.

13. Dr. V. Sreenivasamoorthy, Project Co-ordinator, Microbiology, Fermentation and Sanitation Discipline, Central Food Technological Research Institute, Mysore-13.

14. Dr. Donald T. Wicklow, Microbiologist, Culture Collection Research, Fermentation Laboratory, NRRL, Peoria, Illinois, U.S.A.

15. The Manager, Food Corporation of india, Madras and New Delhi.

16. The Manager, Tamil Nadu Clvil Supplies Corporation, Madras.

17. The Managing Director, Tamil Nadu Poultry Development Corporation (TAPCO), Madras.

Contents

Survey for the presence of Mycotoxins with special reference to Patulin, Sterigmatocystin, Ochratoxin and Penicillic Acid in Foods, Food Products, Animal Feeds and concentrates available in the Tamil Nadu Region of India.

FINAL REPORT

1. (a) Name of the Institution	:	Tamil Nadu Agricultural University, Coimbatore-641 003, India
(b) Department	:	Department of Food Technology.
(c) Location of the Experimental Work	:	Department of Food Technology, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore-641 003, India
2. (a) Sanction No. and Date	:	No. HCS/DST/17/76 dt. 13-7-76.
(b) Sanctioned by	:	Department of Science and Technology, Government of India.
3. Total Grant Sanctioned	:	Rs. 1,92,400-00
4. Total Amount Spent	:	Rs. 1,75,298-43 (Detailed in Tables I and II on pages 3 and 4)
5. Report Period	:	1-8-1976 to 31-7-1979.
6. Report No.	:	6 (Final Report)
7. Date of Start	:	1-8-1976.
8. Date of Closure	:	31-7-1979.
9. Objectives	:	To screen samples of foods, food products, feeds and concentrates from the different districts of the Tamil Nadu region for the presence of Patulin, Sterigmatocystin, Ochratoxin and Penicillic acid; to forecast the toxins present in the food materials and to create an awareness among the public so that consumption of toxic foods by people can be avoided.

10. Principal Investigator : Dr. S. Neelakantan,
Professor and Head,
Department of Food Technology.

11. Other Research and Supporting Staff :

S. No.	Name & Designation	Scale of Pay	Remarks
		Rs.	
1.	Thiru R. Swaminathan, Research Assistant in Microbiology	400-15-430-20-550-25-700	Joined on 1-8-76; Resigned w.e.f. 1-9-78
2.	Dr. (Tmt.) Theymoli Balasubramaniam, Assistant Professor	700-40-1100-50-1600	Joined on 9-8-76 as Research Assistant, Upgraded as Assistant Professor from 1-12-77
3.	Selvi R. Balasaraswathi, Assistant Professor	700-40-1100-50-1600	Joined on 17-11-76 as Research Assistant Upgraded as Assistant Professor from 1-12-77
4.	Tmt. G. Indira Jasmine Aruldass, Assistant Professor	700-40-1100-50-1600	Joined on 23-4-77 as Research Assistant Upgraded as Assistant Professor from 1-12-77
5.	Thiru R. Jayabalan, Laboratory Assistant	280-5-320-10-450	Joined on 1-8-76

Signature of the Principal Investigator

Name: Dr. S. Neelakantan,
Designation: Professor and Head,
Department of Food Technology
Tamil Nadu Agricultural University,
Coimbatore

Signature of the Director of Research of the Institute

Dr. V. Rajagopalan,
Director of Research,
Tamil Nadu Agricultural University,
Coimbatore

Table No. 1

Financial Statement for the Period from 1—8—1976 to 31—7—1979

Name of the Scheme : Survey for the presence of mycotoxins with special reference to Patulin, Sterigmatocystin, Ochratoxin and Penicillic acid in foods, food products, animal feeds and concentrates available in the Tamil Nadu region of India.

Period : 1—8—1976 to 31—7—1979

Grant Sanctioned

1976	: Rs.	28,000.00	
1977	: Rs.	56,000.00	
1978	: Rs.	56,000.00	
1979	: Rs.	52,400.00	
Total Grant	:		Rs. 1,92,400.00

Expenditure

I. Salaries and allowances :—			
Pay	Rs.	73,951-30	
Dearness Allowance	Rs.	11,356-72	
Addl. Dearness Allowance	Rs.	8,841-93	
Non Rec. Ad hoc Relief	Rs.	85-00	
House Rent Allowance	Rs.	5,670-56	
City Compensatory Allowance	Rs.	2,976-60	
Interim Relief	Rs.	2,391-00	
Travelling Allowance	Rs.	4,658-27	
Medical Allowance	Rs.	1,333-00	
Total Salaries & Allowances			Rs. 1,11,264-38
II. Contingencies :—			
1. Recurring	Rs.	59,166-45	
2. Non-Recurring	Rs.	4,867-60	
Total Contingencies			Rs. 64,034-05
Total Expenditure Incurred			Rs. 1,75,298-43
Balance			Rs. 17,101-57

Table No. 2

Yearwise Break-up Details of Expenditure

	1976—77 (1.8.76 — 31.3.77) Rs.	1977—78 (1.4.77 — 31.3.78) Rs.	1978—79 (1.4.78 — 31.3.79) Rs.	1979—80 (1.4.79 — 31.7.79) Rs.	Total
Pay	8,171-42	21,036-80	22,796-79	21,946-29	73,951-30
Dearness Allowance	1,589-62	3,861-90	3,435-00	2,470-20	11,356-72
Addl. Dearness Allowance	1,270-63	3,791-90	3,377-40	402-00	8,841-93
Non. Rec. Ad-hoc Relief	—	—	85-00	—	85-00
House Rent Allowance	817-87	1,372-47	2,266-22	1,214-00	5,670-56
City Compensatory Allowance	408-58	1,051-95	1,133-07	383-00	2,976-60
Interim Relief	—	706-00	1,580-00	105-00	2,391-00
Travelling Allowance	259-65	1,378-65	2,276-37	743-60	4,658-27
Medical Allowance	—	414-65	664-05	254-30	1,333-00
Recurring Contingency	11,219-04	21,797-60	17,733-26	8,416-55	59,166-45
Non-Recurring Contingency	3,009-48	1,858-12	—	—	4,867-60
Total :	26,746-29	57,270-04	55,347-16	35,934-94	1,75,298-43

Introduction and Review of Literature

Mycotoxins, the toxic metabolites of fungal growth, have been in existence since the earliest of times. An outbreak of "turkey-x" disease occurred in England in 1960, due to an aflatoxin containing peanut meal used in the feed of turkey poults. This resulted in the loss of millions of young turkey poults (1). Since then, attention has been focused on mycotoxins and more information on their nature and toxicity has been gained in various laboratories all over the world.

Mycological contamination of agricultural commodities is now being viewed with serious concern. Even then, in our country, the presence of moulds in foods has been given importance mainly from considerations like storage loss and commercial points of sale like colour, taste, etc. During the entire post-harvest periods of storage, food crops are highly susceptible for mould attack. The fungal growth is usually accompanied by the simultaneous production of the toxic metabolites. Toxicity syndromes resulting from the ingestion of contaminated foodstuffs have been referred to as mycotoxicoses (2-4). Several cases of mycotoxicoses in farm animals have been reported by various workers (5-7).

Aflatoxins, patulin, ochratoxins, sterigmatocystin and penicillic acid are all important toxins because of the widespread occurrence of their causative moulds and because all these compounds have been shown to be toxic and carcinogenic to mammals (8, 9). These mycotoxins are produced mainly by the species of *Aspergillus* or *Penicillium,* but not necessarily restricted to any one species or genus. Of these mycotoxins, aflatoxins have attracted the major attention of the scientists all over the world. Aflatoxin as a health hazard has been reviewed by Barnes (10). Species differences in sensitivity to aflatoxins have been observed. Most of the animal species tested were sensitive to the toxic effects of the aflatoxin (11-13). Further, in cattle, the ingestion of aflatoxin-containing toxic meal resulted in the excretion of the toxic metabolites, aflatoxin M_1 and M_2 in their milk (14-16). Lancaster (15) has also discussed the presence of these metabolites in the milk of cows and sheep with particular reference to public health.

The carcinogenic effects of aflatoxins have been reviewed by de Luca (17). Shank *et al.* have studied dietary aflatoxin and incidence of human liver cancer in certain municipal populations of Thailand (18). Mycotoxins as the etiological agents in cancer and in primary liver carcinoma in the South Sahara regions of Africa and in cirrhosis have been well documented (19, 20).

In India, outbreak of aflatoxicosis in cattle was recorded by Sastry *et al.* in 1965. This disease involved 24 Murrah buffaloes in Andhra Pradesh and was characterised by loss of appetite, diarrhoea, dullness, ascites, emaciation, anaemia and icterus. Some consignments of groundnut cake were implicated to have been contaminated with the toxin (21). In another outbreak of mycotoxicosis reported at Karnataka by Gopal *et al.* a total of 126 crossbred cattle were involved out of which 58 died. The symptoms included a orexia, loss of condition, depression, ascites, etc. Groundnut cake was the major component in the feed mix. *A. flavus* was isolated from the feed mix and the level of aflatoxin ranged from 1.1 to 25.0 ppm (22).

Aflatoxicosis in poultry involving the death of 2,219 chicks in Karnataka State was reported (23). In a private farm maintained at Kulu Valley, 4,000 rabbits died due to chronic toxicity arising from toxic feed containing aflatoxin (24). In both the cases, autopsies were performed and histopathological symptoms typical to aflatoxicosis were confirmed. Another outbreak involving mortality and lowered egg production in poultry farms in Erode district of Tamil Nadu was reported by Neelakantan *et al.* (25). The feed contained a very high level of aflatoxin and all samples were found to have *A. flavus* spores in the range of 13×10^3 to 145×10^3 per gram.

Aflatoxicosis in humans in India has been recorded from the studies conducted at both National Institute of Nutrition, Hyderabad and Central Food Technological Research Institute, Mysore. An outbreak of the disease during 1974 in man and domestic dogs with a high mortality rate resulting in the death of more than 100 tribals in the Banswada district of Rajasthan and Panchmahal district of Gujarat was reported by Krishnamachari *et al.* (26-28). The disease was identified as resulting from the consumption of maize containing 6.5 to 15.6 ppm. aflatoxin. The important features of the disease were jaundice, rapidly developing ascites, portal hypertension and in severe cases, death.

The possible role of aflatoxin in the etiology of Indian childhood cirrhosis has been evaluated by Amla *et al.* They have attributed the disease in children due to the consumption of peanut protein supplement in their diet, which contained aflatoxin (29, 30). A study undertaken in South Kanara by Sreenivasamurthy of C. F. T. R. I., Mysore has indicated that there was a positive correlation between aflatoxin content of foodgrains and the incidence of liver enlargement in children (31).

Patulin, a toxic metabolite of *A. clavatus* and *P. patulum* has been found to be decidedly toxic to animals (32, 33). Forgacs *et al.* (34) first demonstrated the toxicity of *A. clavatus* when this species together with *A. chevalieri* was implicated in an outbreak of chronic toxicity in calves in U. S. A. fed with contaminated feed. An ether extract of *A. clavatus* grown on bread produced skin inflammation on a calf, and acute and chronic symptoms leading to hyperkeratosis and death when the calf was force-fed. Similarly, in 1952, a loss of over 100 dairy cattle in Japan was attributed by Yamamoto to the consumption of mouldy feeds contaminated with the toxigenic strain of *P. urticae* (35). Moreau and Moreau (36, 37) reported another outbreak in dairy cattle when farmers in France fed their cows with forage seedlings instead of normal rations due to severe drought in 1959. The seedlings were found to be heavily contaminated with *A. clavatus* and the symptoms included fever, incoordination and hepatic degeneration. The phytotoxicity of patulin has been clearly demonstrated by Norstadt and McCalla. It was shown that stubble mulching of fields supported extensive growth of *P. urticae,* which produced patulin, causing reduced wheat seed germination rate and plant size (38). Indeed, wheat seed and seedlings have been shown to be sensitive to as little as 20 ppm. patulin (39, 40). It has been demonstrated to have carcinogenic properties in mice and has been demonstrated to be a carcinogen (8). Patulin and penicillic acid have been detected and estimated in flours and fruit juices by Scott and Somers (41). The subject of patulin as a mycotoxin of potential concern in foods has been reviewed by Stott and Bullerman (42).

Ochratoxins have been isolated from *A. ochraceous* which occurs in a variety of agricultural commodities and have been demonstrated to be toxigenic to ducklings and rats by many workers (43-48). *A. ochraceous* has been found to invade and multiply in stored wheat (49), red and black pepper, etc. (45) and this toxigenic fungi has also been isolated from cereals and legume products (50). Purchase *et al.* have apprehended that the high incidence of hepatic cancer among the Bantus of South Africa may be the result of the toxic metabolites of these moulds (47). Choudry *et al.* have studied ochratoxin toxicity in hens. They found that the presence of ochratoxin at 1 ppm. in the diet of white leghorn pullets resulted in delayed sexual maturity and lowered rate of egg production. With increasing levels, production was reduced further and pullets became emaciated. Liver and kidney damage similar to the aflatoxin injury was seen (51). In toxic potency, it is estimated to have an oral LD_{50} of about 150 mg. per duckling, one tenth the toxicity of aflatoxin (52). Van Walbeek *et al.* (53) have shown that ochratoxins can also be produced by *Penicillium viridicatum.*

Sterigmatocystin has been characterised as a metabolite of *A. versicolar.* This toxin has structural similarities with aflatoxins (54). It was also obtained later, from the strains of *A. nidulans* which commonly occurs in peanuts and fodders.

Because of the widespread occurrence of these moulds and the large amounts of the compound elaborated, sterigmatocystin might be as disconcerting as aflatoxins in food, despite its lower toxicity and carcinogenicity (55). Vander Watt and Purchase have included sterigmatocystin as a carcinogen in the list of mycotoxins causing a high incidence of hepatomas in the Bantus of South Africa. It has an oral LD_{50} dose of 166 mg/kg for male rats and 120 mg/kg for female rats (56). Engelbrecht has described the effects of sterigmatocystin on a primary cell culture. These include progressive cell degeneration, inhibition of mitosis, micellar fragmentation, etc. (57).

Penicillic acid was first isolated in 1913 from cultures of the mould *P. puberulum* (58). It has since been found to be a metabolite of a variety of *Penicillium* and *Aspergillius* species. Penicillic acid has been found to be produced in large quantities by certain species of *Penicillium* viz., *P. puberulum, P. martensii,* and *P. cyclopium.* Corn blight or blue eye disease of corn caused by these species is quite common when high moisture corn is stored at low temperature (59). Pencillic acid is toxic to mammals (60) and has also been found to be carcinogenic to rats by subcutaneous injection (61, 62). It was found by Ciegler and Kurtzman that under suitable conditions, penicillic acid production by blue eye fungi on different agricultural commodities was very much possible. These commodities included white rice, barley, sorghum, oats and wheat. A number of *Penicillium* species implicated in the blue eye disease of corn were tested and 5 of the 16 strains tested were found to produce penicillic acid. The rate of production depended upon the strain, substrate and incubation temperature (63).

Many workers have demonstrated the natural occurrence of penicillic acid in various foods. Thorpe and Johnson (64) tested the commercial corn samples of spring 1973 originating in several midwest counties of USA, where contamination with *Fusaria* was prevalent. Penicillic acid was found in 7 out of 20 of these samples at levels ranging from 5 to 231 mg/kg. The same workers also reported that while analysing 20 commercial dried bean samples which had mold contamination, penicillic acid was found in 5 samples at levels ranging from 11 to 179 mg/kg.

The toxic effects of mycotoxins can be classified under two broad categories. One is the acute toxicity resulting from the ingestion of large amounts of the toxins and the other is the chronic toxicity due to the cumulative effect of ingestion of small amounts of the toxin over long periods of time. Incidences of both types of toxic effects have been reported in experimental and farm animals as well as in human beings (7, 11, 19, 65-67).

In India, except for aflatoxins, there has practically been no systematic survey on the occurrence of other mycotoxins in foods and animal feeds. But, these other mycotoxins viz., sterigmatocystin, patulin, ochratoxin and penicillic acid are equally important since these are proven carcinogens and also because their causative organisms are widely prevalent. The tropical climate of India, with high ambient temperatures and high relative humidity is ideal for the growth of moulds. In most of the rural and urban areas, scientific methods of storage are not being practised. The storage structures are also of poor quality. As a result, chances of gross mould infestation in stored grains are high. The predominant groups of moulds found on stored crops of India are *Aspergilli, Fusaria* and *penicillium* (68). Fungal contamination invariably leads to mycotoxin contaminantion.

After removing the mycelial mat, people do not hesitate to use the fungus affected food, either for themselves or for their animals. The toxic metabolites are harmful both to human beings and to cattle and poultry. There is also the danger of contamination of the animal products like milk, meat, egg, etc. To create an awareness to this problem of fungal invasion and mycotoxin contamination among the people, this survey work was undertaken. The survey included collection of different foods, foodgrains, food

products, animal feeds, oil seed cakes and feed ingredients produced and available in Tamil Nadu and analysis of the same to detect the possible presence of any of these toxins and to assess the extent to which they are contaminated. In order to create an awareness among the public, popular articles were also published in English and Tamil. These are included as appendix.

Materials and Methods

A. Materials :—

Samples of cereals, pulses, oil seed cakes, food products and animal feeds were purchased directly from the markets of different localities of Tamil Nadu. The cereals were however mainly procured from the different public storage and distribution agencies like Food Corporation of India, Tamil Nadu Civil Supplies Corporation, and Central Warehousing Corporation. Some of the animal and poultry feed samples were obtained from Tamil Nadu Poultry Development Corporation (TAPCO) and also from various commercial feed manufacturers. The number of samples collected from each of the above sources are listed in Table No. 3. Samples were collected at random to cover all the districts of Tamil Nadu and the places of collection of these samples are shown in Fig. 1 (map of Tamil Nadu) on Page 13. The types of samples collected in these places are tabulated in Tables Nos. 4, 5 and 6 given at the end of the report.

Mycotoxin Standards

The standard toxins required for the analysis were either got from scientists working in the field either abroad or in India or were also purchased from Makor chemicals, Jerusalem, and from Vallabbhai Patel Chest Institute, New Delhi. Similarly, the standard cultures were procured from different laboratories abroad. The list of toxins and toxin producing cultures obtained are listed in Tables Nos. 7 and 8.

Table No. 3 Sources For The Various Samples Collected

Sl. No.	Source	No. of samples collected
1.	Food Corporation of India Godowns	597
2.	Tamil Nadu Civil Supplies Corporation Godowns	222
3.	Central Warehousing Corporation Godowns	46
4.	Hangar Godowns of FCI	22
5.	Tamil Nadu Poultry Development Corporation (feeds)	4
6.	Feed Manufacturers	23
7.	Markets at Different Localities (direct purchase)	1,762
8.	Local Fruit Market (direct purchase)	36
	Total No. of Samples collected :	2,712

Table No. 7 Mycotoxin standards and their Sources

Mycotoxin standard	Source
1. Aflatoxins	1. Laboratory Biochemicals Unit, Vallabbhai Patel Chest Institute, Delhi-7. 2. Dr. V. Sreenivasamurthy, Central Food Technological Research Institute Mysore-13.

2. Ochratoxins A & B	1. Dr. Nathan L. Brown, Dept. of Health, Education & Welfare Washington D. C. 20201, U.S.A 2. Dr. Richard Cole, USDA, ARS, Dawson, Georgia, U.S A.
3. Patulin	1. Dr. Lloyd Bullerman, Dept. of Food Technology, University of Nebraska, Lincoln, Nebraska, U.S.A. 2. Dr. Nathan L. Brown 3. Dr. Richard Cole
4. Penicillic acid	1. Dr. Meridith, U.S.D.A., NRRL, Peoria, Illinois, U.S.A. 2. Dr. Alex Ciegler, NRRL ARS — USDA Peoria, Illinois, U.S.A. 3. Makor Chemicals, Jerusalem, Israel.
5. Sterigmatocystin	1. Dr. L. Stoloff, FDA, Washington, D.C., U.S.A. 2. Dr. H. W. Schroeder, USDA, College Station, Texas, U.S.A.
6. Other toxins like Zearalenone, citrinin, luteoskyrin etc	1. Dr. C. W. Hesseltine, Chief, Fermentation Laboratory, NRRL, Peoria, Illinois, U.S.A. 2. Dr. Glen Bennett, NRRL, Peoria, Illinois, U.S.A. 3. Dr. Nathan L. Brown 4. Dr. Richard Cole 5. Dr. Stanley Nesheim, Dept. of Health, Education & Welfare, Washington DC., U.S.A.

Table No. 8 Toxigenic Cultures and their Sources

Toxigenic Fungal Culture		Source
NRRL 1958	*Penicillium urticae*	Joseph Lovet, Division of Microbiology, FDA, Washington DC., U.S.A.
M 26	*P. patulum*	
ATCC 9596	*A. clavatus*	
M 1136	*P. urticae*	
NRRL 2300	*P. griesofulvum*	
NRRL 1002	*P. claviforme*	
ATCC 9599	*A. clavatus*	
6139	*A. versicolar*	Dr. H. W. Schroeder, U.S.D.A., Texas; U.S.A.
NRRL 1241	*A. rugulosus*	
NRRL 210	*A. rugulosus*	
	A. flavus	Dr. L. L. Flag, Dept. of Plant Pathology, UPLB College of Agriculture, Phillipines.
NRRL 6318	*P. martensii*	Dr. H. F. Schindler, Dept. of Health, Education and Welfare, Washington DC., U.S.A.
M 1246	*P. urticae*	
M 1238 / NRRL 6316	*P. cyclopium*	
M 298	*A. ochraceous*	Dr. Donald T. Wicklow, NRRL Centre, Peoria, Illinois, U.S.A.
M 1091	*A. versicolor*	
NRRL 3564	*P. puberulum*	
NRRL 942	*P. cyclopium*	
NRRL 3608	*P. martensii*	
NRRL 2040	*P. puberulum*	
NRRL 5864	*F. graminarum*	Dr. Glenn Bennet and Dr. C. W. Hesseltine, NRRL, Peoria, Illinois.

B. Methods :—

I. General

The collected samples were serially numbered and their physical characteristics were observed critically with respect to fungus growth, insect damage, caking, color, appearance and general condition. The data on such characteristics for only those samples which showed positive presence for any of the toxins under reference are tabulated in Table No. 9 given at the end of the report.

II. Chemical Assay

The samples were coarsely ground in a hand grinder. Fifty gms. of the coarsely ground sample were extracted with 200 ml. chloroform in a 500 ml. Erlenmeyer flask on a rotary shaker for one hr. The slurry was filtered through a buchner funnel. The clear filtrate was washed once with 50 ml. of distilled water and then was dried over anhydrous sodium sulphate. The dried chloroform extract was distilled over a water bath under vacuum. The remaining residue was dissolved again in a little chloroform and filtered through filter paper directly into clean and dry small injection vials. Only in those cases, where quantitative assay were required, the concentrated extract was made up to a known volume before spotting on TLC plates. The concentrated extracts of all samples were maintained at — 10°C in a deep freeze until required for analysis.

Standard thin layer chromatographic procedures were followed, using Silica Gel G at a thickness of 0.25 mm. The coated plates were activated at 110°C for one hr. and then stored in a dessicator cabinet. The concentrates were spotted along with pure standards and the chromatogram was developed with a solvent system of toluene : ethyl acetate : 85% formic acid (TEF) in the ratio of 6:3:1 for a distance of 15 cms. For each sample, a set of five plates were prepared. The plates were air dried and then viewed under long wave and then short wave U. V. lamp in a chromatovue cabinet. The various fluorescent coloured spots observed were marked. Identification of the individual toxins was done using various color reagents as shown in Table No. 10 Ammonia fumes were used for detecting ochratoxin; phenyl hydrazine reagent for patulin; aluminium chloride for sterigmatocystin; *p*-dimethyl amino-benzaldehyde reagent and also ammonia fumes for penicillic acid and *p*-anisaldehyde as a common reagent for all toxins. Whenever positive reactions were given by any sample, the results were confirmed by rechromatography of the particular sample extract along with the specific pure toxin. In such cases, usually 4 or 5 spots of the suspected samples were placed on the TLC plate along with standard at different concentrations. Rf values and specific colour reactions were used as confirmatory tests for the toxins. The colour of the fluorescent spot under long/short wave U. V. lamp was also noted. In the case of aflatoxins, the characteristic blue fluorescence at the same Rf value as that of standard toxin was used as the presumptive test. This was followed by rechromatography at different concentrations (minute to large) for confirming this toxin. Quantification of aflatoxin in contaminated feed mixtures was done separately for each sample by spotting different concentrations of the standard and sample solutions and by matching and comparing the intensity of fluorescence of the sample with that of the standard.

Table No. 10 given on page 12 gives the various colour and fluorescence reactions due to the application of specific chemical spray reagents.

III. Mcrobiological Assay

Ten gms. of each of the sample were taken in 100 ml. sterilised water blanks and shaken well in a mechanical shaker for 15 mins. Then serial dilution was made to obtain 1/1000 dilution. One ml. from this dilution was transferred into sterilized petridishes. Czapek's agar medium was poured over into the petridishes and then gently mixed. Three replicates were prepared for each sample. The petridishes were incubated at room temperature for five days. On the fifth day, the number of fungal colonies were counted and tabulated. The fungi were subcultured for further study and identification.

Composition of the Czapek's Agar Medium used

Sodium nitrate 3.00g; Dipotassium hydrogen phosphate 1.00g; Potassium chloride 0.50g; Magnesium sulphate 3.50g; Ferrous sulphate 0.01g; Sucrose 30.00g; Agar 15.00g; Water 1000 ml.

IV. Development of Spray Reagents for the Detection of Penicillic Acid on the TLC Plates

During our preliminary screening work, we had considerable difficulty in visualising penicillic

acid on TLC plates by the usual ammonia fumes or *p*-anisaldehyde spray reagents (71, 59). Consequently it became necessary to develop alternate procedures specific for penicillic acid. We found that *p*-tolualdehyde (TA) and *p*-dimethyl-aminobenzaldehyde (DABA) gave brightly coloured spots with penicillic acid under visible and long wave U.V. light.

Standard TLC procedures were followed using activated TLC plates and TEF solvent system as described above. The aldehyde spray reagents were prepared with the following composition: TA or DABA: concentrated sulphuric acid: acetic acid: methanol, 1:5:10:70. Penicillic acid was dissolved in chloroform and spotted at various concentrations between one and five ug. After the chromatography, the plates were allowed to dry and the aldehyde colour reagents were sprayed. After spraying, the plates were heated and maintained at 130°C for 5-8 mins. when distinct bright coloured spots developed. However, before heating, no visible colour could be seen. The colours were compared with Horticultural Colour Charts (73.).

Table No. 10. Rf value, Colour Reagent and Confirmatory Tests for Different Mycotoxins

S. No.	Reagent	Toxin detected	R_f value	Colour/ fluorescence formation	UV/visible light	Ref
1.	Ammonia fumes	Ochratoxin A	0.55	Green to blue	UV	126
		Penicillic acid	0.47	Blue	UV	59
2.	4% Phenyl-hydrazine in 75% ethanol	Patulin	0.41	Yellow	Visible	69
		Penicillic acid	0.47	Green	UV	
3.	20% Ethanolic Aluminium chloride	Sterigmato-cystin	0.85	Yellow	UV	70
4.	*p*-Anisaldehyde	Ochratoxin A		Faint blue fluorescence (difficult to observe)	UV	71
		Patulin		Yellow	UV	
		Penicillic acid		difficult to observe green in visible light		
				gr. blue	UV	
5.	*p*-Dimethyl -aminobenzal-dehyde	Penicillic acid		Yellow	UV	72
				Pink on spraying with ethanol	Visible	

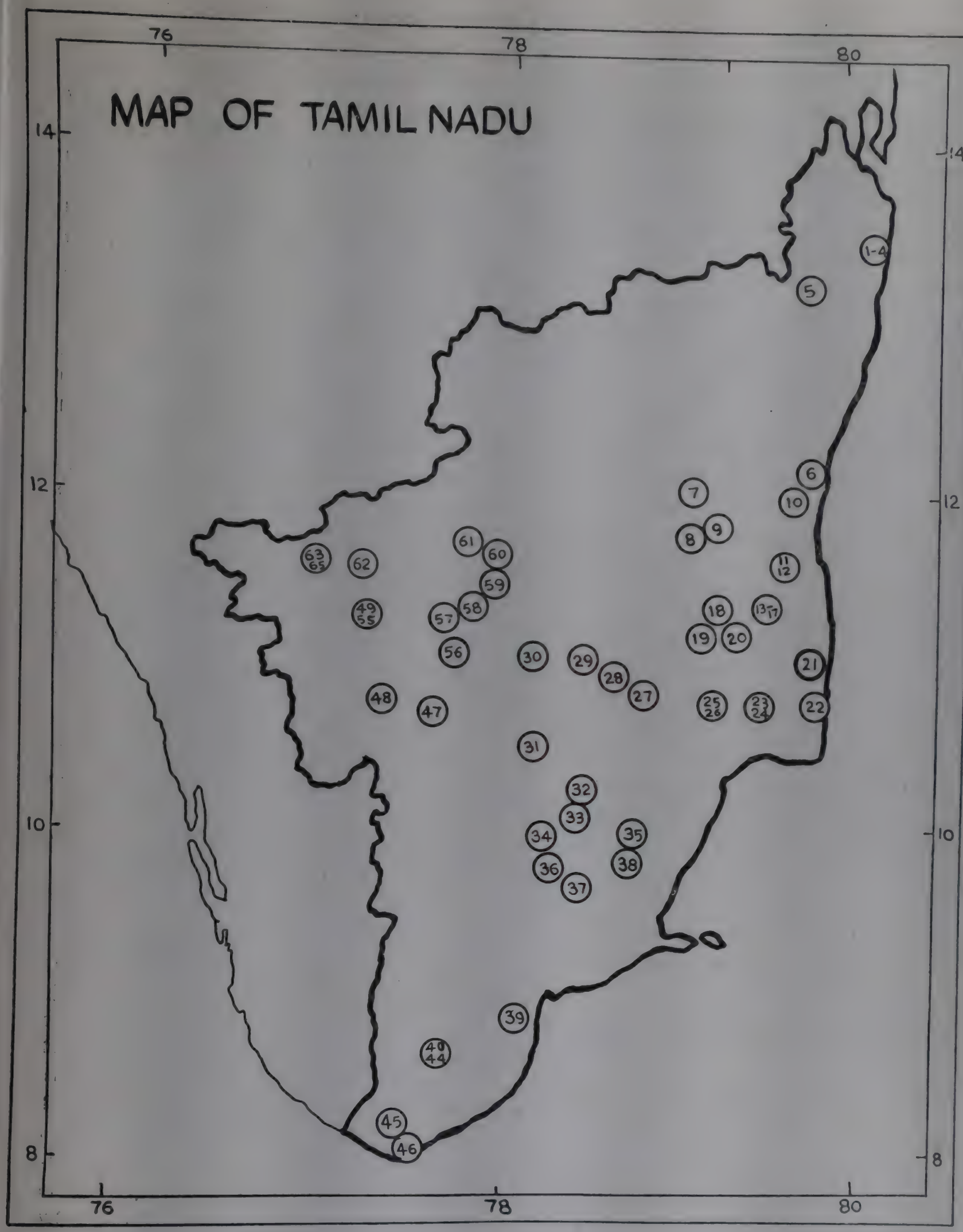

Fig. 1. Map of Tamil Nadu showing places of collection of samples

Results and Discussion

During the period of three years, a total number of 2,712 samples were collected from different areas of Tamil Nadu State. The collected samples included the following items: wheat (528*) maida (10), rava (7), rice (360), pounded rice (12), sorghum (12), samai (2), bajra (1), maize (1), milo (1), toor dhal (122), bengal gram dhal (119), urad dhal (106), green gram dhal (23), pea dhal (7), mixed dhal (3), green gram (33), beans (32), pea (24), black gram (17), bengal gram (17), red gram (16), horse gram (10), cow pea (15), papads (130), vermicelli (47), bakery products like bread, bun, cakes (15), varki (5), biscuits (11), gingely candy (9), kammarkat candy (9), groundnut candy (91), copra (6), dried grapes (52), dried dates (18), dried vegetables (43) pickles (37), jam (3), apple juice (1), scented supari (67), arecanut (54), arecanut flakes (26), miscellaneous food products (18), groundnut, oilcake (134), cocount oilcake (96), gingelly oil cake (83), oil seed cake mixture (15), cotton seed oil cake (4), bran (87), poultry feed (60), cattle feed (38), cotton seed and husk (30). Besides, horticultural produce like fresh fruits viz, papaya (9), tomato (7), apple (6), banana (5), grapes (3), orange (3), lime (1), gooseberry (1) and cluster apple (1) were also collected and analysed.

On chemical analysis of all these samples, we found that 4.13 per cent of the collected samples were contaminated with any one of the mycotoxins under reference. The break up details are given in Table No. 9 given at the end of the report.

Chemical Identification of the Toxins:

I. Sterigmatocystin in Grain Samples

The presence of sterigmatocystin was detected in twenty foodgrain samples of rice and wheat. The rice included both raw and parboiled types and wheat included domestic and imported red and white wheats. These foodgrains were collected from various godowns belonging to different agencies who store grains for the public distribution system. These different agencies are shown in Table No. 3 along with the number of samples collected from them. The survey included most of the important centres in Tamil Nadu State. As can be seen from Table No. 9, most of those samples showing positive presence for sterigmatocystin were highly mold infected, discoloured and caked up. They were generally unfit for human consumption. However, even a few normal looking samples also showed the presence of this toxin. But we do not know the previous history of these samples. The samples were collected at random from various godowns located at various places which have varying environmental conditions of temperature and humidity. The coastal areas have high humidity compared to inland districts and temperature difference of about 10°C at different places. Wheat sample Nos. 627, 628 and 794 and Rice sample No. 752 were collected from open air cap storage systems located at Sozhavaram and Trichy. Samples collected from these systems were invariably affected by the rains at one time or the other inspite of many precautions taken. Many of the samples would have been stacked for more than one year though given fumigation treatments as a routine by the warehousing staff. Only the highly discoloured and caked up grain were segregated and were kept separately in the warehouse as unfit for human consumption.

Sterigmatocystin was detected by Scott *et al.* in one of the twenty nine samlpes of heated wheat grains from a Saskatchewan farm (74). The grains had got discoloured. Purchase has reported that samples of coffee beans were found to be contaminated with this toxin (75). It was found that Sterigmatocystin was found as a natural contaminant in barley grains meant fo feed purposes in U. K. (76). Studies have shown that sterigmatocystin producing fungi were present in moulded samples of dried beans (77), country cured ham (78) and in smoked meats (79) although the actual presence of the toxin was not demonstrated. Sterigmatocystin is produced as a toxic metabolite by the common molds viz, *A. nidulans, A. rugulosus, A. versicolar, Bipolaris sorokiniana* and *P. luteum* (80). *A. unguis* (77)

(*The number in brackets denotes the total number of samples for each category)

was also found to produce this toxin. There is a high possibility of contamination by some of these moulds in our stored grains. It has also been reported that *A. versicolor* and *A. ochraceous* may have a relatively high incidence in commodities due to their relatively low moisture requirements, since their conidia can germinate at a relative humidity of 81% (77).

Manabe (81) has reported that sterigmatocystin contamination was detected in 12 out of 37 rice samples while studying the mycological damage of domestic brown rice during storage in warehouse under natural conditions. All the 12 samples were obtained from the lowest position near tne floor in the lot examined. The highest contamination value obtained was 16.3 ppm. He concluded that the brown rice stored at the lowest position in a warehouse must be very carefully handled from the point of view of preventing sterigmatocystin contamination. There has been no previous report of sterigmatocystin contamination of food commodities in India.

II Sterigmatocystin in Cotton Seeds

Sterigmatocystin was also found in two samples of cotton seed, (Sample Nos. 1,428 and 1,429) purchased from the market at Ooty (The Nilgiris district). The general condition of the samples was good. Usually, it is difficult to recognise mould growth on cotton seeds, because of the presence of lint over them.

Cotton seed is generally used for oil extraction and this oil is used for the manufacture of vanaspathi by refining and hydrogenation. The cake is used as a cattle feed and for compounding animal feeds. The place of collection of the sample is at an altitude of 2,100 metres above the mean sea level and the place remains generally cool throughout the year with high ambient humid conditions. No information of the previous history of the sample was available. Since the cotton seed sold in the markets are mainly meant for dairy cattle, dairy fa mers whether big or small purchase the same for increasing the milk yield. Since sterigmatocystin is a toxin closely related to aflatoxin, it is capable of causing severe damage to the vital organs of the cattle and also pose severe health hazard to the human beings. In an earlier report, Raghavendra Rao *et al.* (82) have reported contamination of Indian cottonseed with various *Aspergillus* species. In a survey of cotton seed samples obtained from various places of India, they observed that in dry regions, 46.3% of the samples was found to be infected with *A. nidulans* and 1.6% of the samples were contaminated by *A. versicolor.* In humid regions, the figures were 48% and 1.6% respectively. It has been well documented that these *Aspergillus* species are capable of producing sterigmatocystin under varying experimental conditions on different agricultural commodities (55).

The tropical climate of India, prevailing with high relative humidity and ambient temperatures offers excellent conditions for the mould growth. Scientific methods of storage are not practised in most of the rural as well as urban areas. Even organised warehousing agencies lack sufficient facilities to safeguard the grains against mould attack. It has been reported that in 1977–78, over 80 million rupees worth of foodgrains were spoiled in this country because of ineffective and inadequate storage (83). Since millions of tonnes of foodgrains have to be stored by these agencies, top most priority has to be given in our Development Plans for increasing the country's storage facilities keeping in view of our rapidly increasing food production.

III Penicillic Acid in Apple

Penicillic acid was detected in one sample of apple (Sample No. 1,965), having green mould growth on it. This was purchased from the local market. The rot had set in and this sample on analysis clearly showed penicillic acid to be present. This was later confirmed by the characteristic coloured spots on TLC plates, by spraying with TA and DABA. (84). A total number of six samples were analysed.

Bullerman (85) surveyed 11 brands each of yellow and white popcorn for the condition known

as 'Blue Eye'. Screening of the mould isolates for mycotoxin production revealed that 84.4% of the *Penicillium* isolates were capable of producing penicillic acid. *P. martensii* was found predominating in the kernels. No penicillic acid was detected in the blue eyed popcorn kernels as such. However, after cracking and incubating for 10 days, they were found to contain detectable amounts of penicillic acid.

Mislivec and coworkers (86) found that the mould flora of 75 commercial samples of dried beans was dominated by species of *Aspergillus* and *Penicillium* including *P. cyclopium.* They reported that *P. cyclopium* isolates when grown on sterile rice produced penicillic acid.

The culture extracts of the mould isolates obtained from mouldy cheddar cheese were examined by Bulleman and Olivigni for their production of mycotoxins on yeast extract sucrose or potato dextrose broth. Penicillic acid was detected in most of the extracts along with patulin and ochratoxin (87). Similarly Swiss cheese samples that developed visible mould growth after six weeks at 5°C were analysed. Penicillic acid appeared to be present in 4 of 33 of the samples. The quantity of the toxin in cheese trimmings estimated by visual comparison on TLC plates was 0.5 μg/g of cheese. Toxicological screening of the mould isolates showed that they were capable of producing penicillic acid on Yeast Extract Sucrose broth (88).

Thorpe and Johnson (64) analysed seven samples of apple juice obtained from bruised and rotten apples and twelve samples of commercial apple juice collected from stores in the Washington D.C. area. They could not detect penicillic acid in any of these samples.

In the present case, the apples had developed visible greenish mould growth on the surface and the rot had set in. We have not been able to locate previous literature on the occurrence of penicillic acid in apples and possibly this is the first time that this toxin has been reported to be occurring in apples.

Generally, such apples are graded low by the vendors with substantial reduction in the price and they are generally purchased by the poorer sections of the public. Rotting and moulding of fruits generally start with bruising on their surface during harvesting and transportation. Generally, apples are cold stored and when they are transported to the market, moisture condenses over their surface creating a congenial atmosphere for moulds to develop. There are no refrigerated transportation or refrigerated display facilities available in India. If the produce is handled carefully during its entire post-harvest stages of storage, transportation, and retail display, much of the physical damages and bruises can be avoided. Bruising exposes the cell sap for quick microbial attack. Since such rotting and moulding cannot be prevented, it is essential that proper packaging should be practised by the merchants at various levels of marketing.

IV Patulin in *Scented Supari*

One sample of scented *supari* (processed arecanut) (Sample No. 2,318) was found to contain patulin. This sample was purchased from a shop in Nattarmangalam of South Arcot district. The appearance and general condition of this particular sample was found to be good. The scented *supari* was purchased in unit sized consumer packets of 2-3g. These are packed in wax paper. This is how scented *supari* is predominantly marketed in South India. During high humidity conditions, there are chances for moisture penetration and development of mould growth. Further, arecanut as such, is also known to be susceptible for fungal invasion, unless properly dehusked and dried immediately after harvest. Scented *supari* is prepared by various commercial processes which involve boiling, and steeping in concentrated arecanut extracts *(kali).* Since various artificial flavorings, spices, colour, etc. are added before packing, any bad smell or colour due to mould growth will be found generally masked. It is felt that toxin production may not occur after processing. We feel that the patulin development is mainly from the original mouldy raw material, used in such specific cases. We are not aware of patulin having been reported to be present in arecanut samples by any other laboratory.

It appears to us, that possibly this is the first time scented *supari* has been indicted to contain patulin in detectable amounts. *Supari* is chewed, generally by vast sections of our population in rural and urban areas, by the poor as well as the rich people. At various times, it has been found that the contents of the packets had been caked up with diffuse mould growth on them. Usually, this occurs during storage at the retail level under adverse conditions. Since we find that *supari* can also be a substrate for the production of mycotoxins, it arouses an anxiety in us that such damaged samples are not used for further processing. It is very difficult to detect damage after processing, unless fresh mould growth occurs in the consumer sized packets during storage. It is quite possible that mouldy nuts are easily diverted by the trade for the production of scented *supari* since no bad effects can be detected. Unscrupulous utilization of mouldy nuts for the production of *supari,* might cause the toxin to be diffusedly present in a large number of packets, since it ts broken into smaller particles and mixed. We feel there is ample scope for further work in this direction with respect to *supari* and we feel that more survey work on this material should be taken up.

Various workers have reported the isolation of patulin proudcing moulds from a variety of commercial food products and poultry feeds. These include cereal grains and legumes (89); inshell pecans (90), apricots, crab apples and persimmons (91), pears and grapes (91, 92) and apples (92, 93, 94) Swiss cheese (88), cheddar cheese (87) etc. However, apple and apple products have been found to be the foods most commonly to be contaminated with patulin. Sixtysix percent of naturally rotted apples yielded *P. expansum* isolates which were observed to produce patulin (93)*

Though patulin producing moulds have been found on a variety of foods, only apple sap, apple juice and apple cider have been found to be naturally contaminated with this toxin (91, 93-98). There is a growing concern about the occurrence of this toxin in foods and many countries are anxious to set tolerance limits for this toxin in fruit juices. Norwegian Ministry has provisionally decided that 50 μg/ litre is the highest acceptable content of patulin in apple juice (99). Sweden has set an action limit of 60 ng/ml for patulin in fruit juices (100). Trimming of rotten tissues from apple was found to effectively remove 93-99% of the total patulin content regardless of fungus strain, incubation temperature or apple variety (101). Thus it was found that trimming of rotten portions could substantially reduce the patulin concentration. There is a recent report in which Finnish investigators have found patulin in 21 of 23 spontaneously moulded domestic bread samples (102).

V Aflatoxin in Various Commodities

A. General discussion on the occurrence of aflatoxin in foods and feeds :

It appears from the scanning of literature, that almost any type of food can harbour toxigenic strains of *Aspergillus* which can produce aflatoxins under suitable conditions and on proper substrates. These include cereals, legumes, fresh horticultural produce, dairy and meat products etc. Possibly such contamination was there from time immemorial. The modern analytical techniques have far advanced that we are able to detect, isolate and identify micro quantities of the toxin. Mould contamination of foods cannot be considered any more merely as an aesthetic problem or only from the point of view of 'freedom from faith'. Studies have clearly established aflatoxicosis in farm animals and humans. In India, the problem has serious connotation, because, majority of our people are poor, illiteate and their nutritional status is much below normal standards. In developed nations, a few control measures have been taken to prevent a large scale ingestion of the toxic foods and feeds by their people and farm animals.

A German ordinance effective March 1, 1977 prohibits the use of foods when aflatoxin B_1 concentration is more than 5 ng/g or total aflatoxins are more than 10 ng/g (100).

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*In a survey of apple juice in U. S. markets, 37% of the samples tested were found to contain detectable amounts of patulin ranging from 40-440 μg / litre. (94)

Switzerland has imposed the same restrictions on various imported nuts, fruit kernels and other edible seeds, which are being tightened to 1 ng/g as the average of 10 samples from a lot for aflatoxin B_1 or 5 ng/g for total aflatoxin (100).

Austria has banned outright importation of Brazil nuts because of the possibility of aflatoxin contamination and has set a total aflatoxin limit of 5 ng/g for various nuts, edible seeds and grain (100).

European Economic Community have fixed maximum permissible limits for aflatoxin B_1 from 10 to 50 ng/g in mixed feeds depending upon the type of animal for which the feed is intended (103).

U. S. A. has set a tolerance limit of 15 ppb for aflatoxin in shelled peanuts and peanut products used as human food (101). Canadian Health Protection Branch has set an actionable level for aflatoxin in nuts and products at 15 ppb (105).

Thus we find that there is an awakening and concern over mycotoxin contamination in general and aflatoxoin in particular, in many countries. Since prevention is better than cure, it is felt that there is an urgent need in our cuntry to create an awareness towards this problem amongst the producers, public warehousing agencies, governmental machineries, trading community and consumers. All these mycotoxins have been shown to be toxic, in the animal systems in various ways. Some are powerfully hepatotoxic, carcinogenic, mutagenic, teratogenic, and some can affect the victal hepatic, nervous or renal functions of the body depending on the toxin.

During the first year of the survey, analysis of cereals and other grains were undertaken and our attention was focused mainly on toxins other than aflatoxin. However, during the second and third year of the survey, when analysis of oil seed cakes were undertaken, aflatoxin was seen to be present in some of the samples. Hence, it was made a regular practice to look for this toxin also. Survey for aflatoxins in different commodities have been reported from various research laboratories (82, 106-109). In our studies, we have analysed various food products meant for human consumption as well as oil seed cakes, and animal feeds. The detailed discussion on the presence of aflatoxin in these commodities is given below. We could not quantify the toxin content in these samples.

B. Cottonseed :

Thirty samples of cotton seed were analysed and two samples out of these thirty were positive for aflatoxin. Both samples were found to be discoloured on inspection, and they contained both aflatoxin B_1 and B_2. Generally, the consumers look for discolouration and bad odour as index of spoilage and unless the price is very much reduced, such samples may not be purchased by the people.

It has been well documented that cottonseed is a good substrate for the growth of various *Aspergillus* species and it supports the production of aflatoxin (82, 110-113). As discussed earlier, cottonseed as well as its cake are used as dairy cattle feeds and its protein concentrate is also considered for large scale supplementation of human diet in various parts of the world. It is also well known that these aflatoxins are secreted in the milk in the form of derivatives as aflatoxin M_1 and M_2 which can lead to serious consequences. Again, this study has highlighted the importance of proper post harvest storage and handling of materials in preventing mould growth.

C. Groundnut Oilcake :

It is well known that aflatoxin is a serious contaminant in groundnut oilcake. Out of 134 samples of this item analysed, twentyeight samples recorded positive presence for aflatoxin. Approximately, one out of every five samples of groundnut oil cake contained aflatoxin. The contaminated samples are from almost all the districts of the State. Of these twentyeight samples, fifteen of them contained both aflatoxin B_1 and B_2, while the rest of them contained only B_1. These toxins are produced by the moulds of the *Aspergillus*

species, especially *A. flavus* and *A. parasiticus*. It is now clearly established that the fungus grows on groundnuts when they are stored after harvest without adequate drying. In India, it is the usual practice for the farmers to wait for a shower of rain before harvesting the groundnuts so that the soil becomes loose and the plants could be easily pulled out. Consequently the moisture level in the crop rises and the humidity also increases. Further, proper drying yards or machineries are not available to dry the harvested crop. So the nuts become susceptible for mould growth with consequent toxin production. The processing of seeds is oriented only for the production of oil for human consumption. The oilseed cake is considered only as a minor by-product. No manufacturer cares to remove the infected seeds before crushing and so the problem of aflatoxin in groundnut and its products is a very serious one in our country.

Afltoxins B_1 and B_2 have been found to be regular contaminants in unrefined peanut oil obtained in the Indian market at levels of 0.02 to 0.2 ppm (114). The oilseed cakes are mainly used for dairy cattle feeding as protein concentrates. There is no control over quality as the oil mills just dispose of the cakes to local traders who sell them as such or (after mixing with other ingredients as animal feeds).

A total of 298 peanut pod samples and 92 cake samples of the rabi season and 445 pod-samples and 49 cake samples of *Kharif* season were screened by Rao *et al.* for aflatoxin. The levels of *A. flavus* fungal contamination and the levels of toxin were found invariably markedly higher in the *Kharif* season (115). Another survey in 1965-67 on peanuts and cakes involving 600 samples showed greater contamination in samples drawn from Andhra and Tamil Nadu regions. About 82% of the oil-cakes contained aflatoxin at fairly high levels (116). In another study, more than 50 percent of the 97 groundnut cake samples drawn from Madhya Pradesh region were found to be positive for aflatoxin (117). In a latest survey, seventy percent of groundnut cakes sold in the market of Hapur of Uttar Pradesh region of India were found to be contaminated with aflatoxin. The toxin content were found to be very high, ranging between 1135 to 2250 ppb (118). Countries importing groundnut cake from this country appear to have fixed a limit of 60-120 ppb. An E. E. C. Council directive provides for maximum allowable limits of aflatoxin B_1 in mixed feeds. This directive which is binding on all 9 EEC countries, sets maximum levels from 10 to 50 ng/g. depending upon the type of animal for which the feed is intended (103). Thus aflatoxin in groundnuts constitute a serious health hazard directly through the consumption of contaminated oil and indirectly through the use of its cake in animal feeds.

D. Gingelly Oilcake :

Out of a total number of eightythree samples analysed, four samples showed the presence of aflatoxin. Like any other oilseed cake, it is very difficult to judge the presence of fungi on these cakes. Hence, the possibility of fungal contamination and concomitant elucidation of the toxin in these materials would have taken place in the oilseeds themselves before pressing them for oil. Out of these four contaminated samples, three of them contained aflatoxins B_1 and B_2 while one of them contained B_1 only. It appears that even gingelly oilcake is a good substrate for the production of aflatoxin. We are not aware of any published report of aflatoxin contamination in this commodity and this could be the first report of aflatoxin in gingelly oilcake. We feel more work is required in this commodity.

E. Coconut Oilcake :

Ninetysix samples were purchased from different parts of Tamil Nadu, wherever it was commonly available. It was found that three of these samples were positive for aflatoxin B_1 only. Except for one sample, which was found to be slightly mouldy, other samples were good in appearance without any visible defects.

It is not uncommon that the coconut oilcake finds its way in the coconut chutney preparations in hotels as a substitute and/or as an adulterant for coconuts. So there is a good chance

for this item to get into the dietary of the public indirectly through these unscrupulous practices. The coconut, being a nutritionally efficient medium, heavy mould contamination is possible, whenever there is delay in drying due to rains and/or due to the prevalence of high relative humidity in the drying areas. Occurrence of heavy mould growth during drying of the broken coconuts, before oil extraction, is a common sight in coastal areas. The period of infection of the copra would be during the process of drying and curing, as it is done by drying in the sun on large open mats. The moisture, high nutritive value of the coconut pulp and the thin film of coconut water covering it, act as excellent medium for the growth of micro organisms (119).

Coconut oil is also used widely in places where coconut is grown extensively. Since it is well known that aflatoxin gets distributed between the cake and the oil in the case of groundnut oil manufacture, the same is true of coconut oil too. It has been reported that out of 10 samples of coconut oil analysed 4 samples showed the presence of aflatoxin in the range of 0-20 μg/kg (106).

F. Brans :

Brans obtained while processing cereals, millets and pulses contain besides fibre, small amounts of protein and vitamins. The brans thus obtained are invariably used for feeding cattle and poultry as a routine practice. Eightyseven samples of such brans were purchased from different locations and on analysis we found that none of these samples contained mycotoxins, especially aflatoxin. It appears that possibly such brans do not support the production of aflatoxin, as they are not generally rich sources of readily usable carbohydrates.

G. Manufactured Feed Concentrates :

Nowadays, the market is flooded with a large number of feed concentrates under different trade names. These usually are supposed to contain a full complement of protein, vitamins, minerals, etc. obtained from a variety of sources like oilseed cakes in large proprotion, brans, fish-meal, etc. Thirtyeight such feed concentrates were analysed and it was found that seven of them were contaminated with aflatoxin. Of these, five contained B_1 only, while two of them contained aflatoxins B_1 as well as B_2. Some of these samples were sent by the veterinary surgeons as "suspected" and invariably they contained a huge amount of aflatoxin. For example, sample No. 1436 contained 2400 μg/kg and sample Nos. 1058 and 1059 contained 1200 μg/kg each of aflatoxin B_1. The animals fed on this feed were going down in condition and appeared emaciated. Three of the animals died. They showed liver lesion at the postmortem examination. Histologically there was, toxic liver damage and the lesions were similar to those seen in aflatoxicosis in cattle (120).

Since mould growth as such is not commonly seen in such established feed samples, the dairy farmers may not be able to identify them and they go mainly by the brand names. Since more than 15% of such samples were found to be contaminated with aflatoxin, we feel that there is a strinngent need for adopting quality control measures by the feed manufacturers. There should also be an effective government controlled monitoring agency like in other countries. There appears to be no official regulatory mechanism for animal feeds in India. Many feed manufacturers do not have a separate quality control system, with the result, many contaminated samples have scope to enter the market. Since these feeds are manufactured from different ingredients mentioned above, and since they are purchased from different outside sources, it is essential that feed manufacturers shuold have careful watch over the quality of the raw materials. The health hazards caused by aflatoxins, either directly or through the animal system have been well documented by a large number of workers.

H. Oilseed Cake Mixtures :

In certain parts of Tamil Nadu, the local shop keepers sell a mixture of different oil seed cakes in different proportions for animal feed purposes. Out of ten such samples analysed, four were found to contain aflatoxin B_1. Like the other oilseed cakes, it is also difficult to identify the mould

growth on such mixtures. Obviously, the mould contamination should have taken place before pressing for oil. Since many types of oilseed cakes are used for such purposes, extreme care should be taken while using these items also. The general discussion as given above for groundnut oil cake holds good for these mixtures as well.

I. Poultry Feeds :

There is a big business in the poultry feed manufacture at present in our country and this has vast scope for expansion with the establishment of more and more poultry farms. Unlike animal feeds, poultry feeds also contain energy sources besides proteins, vitamins and minerals. These feeds are also manufactured from a large variety of ingredients like oilseed cakes, cereals, millets and their brans, fish meal, etc.

During the latter part of 1977, incidences of higg mortality of poultry birds and a heavy drop in egg production were reported in several poultry farms from an area around Erode in Tamil Nadu. The post-mortem examination of the affected birds revealed toxaemia and enlargement of liver (121, 122). Twenty samples of poultry feed were obtained from this area and on analysis eight samples were found to contain aflatoxin at a concentration of 1000 μg/kg, while two samples had 500 μg/kg and seven showed 200 μg/kg or less of the toxin. Only three samples were free from the toxin. In the positive samples eight samples showed the presence of both B_1 and B_2, while nine samples contained only B_1. In all the positive samples, we could isolate *A. flavus* as the principal mould contaminant. The mould spore count indicated that the load ranged from 13×10^3 to 145×10^3 *A. flavus* spores per gram of the sample (25).

While continuing the survey, forty more feed samples were collected from different parts of the State and analysed. Of these, two samples showed the presence of aflatoxin B_1. The presence of mycotoxins in the feeds causes severe damage to the birds and only when heavy losses occur, the poultry farmers think of getting their feeds analysed. Otherwise, no special precautionary measures are generally taken by them. Incidences of mycotoxicoses in poultry due to contaminated feed is widely prevalent in India. Many times they go unnoticed and proper reporting is also not being done. Extreme precautionary measures have been taken in developed countries to check for the presence of aflatoxin in feeds and rations. On the contrary, foods contaminated and unfit for human consumption are easily diverted for animal and poultry feeds. This can seriously affect the productivity of the animals and may end up in severe economic loss besides causing health hazards to our population. Aflatoxin B_1, has been found in both yolk and white of eggs produced by hens receiving aflatoxin experimentally in their rations (123). An awakening in this direction amongst the feed manufacturers is essential.

J. Prepared Food Products :

i) Groundnut Candies :

It is one of the very common snack items, that is eaten with relish by both young and old throughout India. It is usually prepared by making bars or balls of roasted groundnut and thick syrup. These are not costly and so they are purchased by people of all sections of the society. We had analysed 91 samples of this item, out of which four were found contaminated with aflatoxin. While two of them contained only B_1, the remaining two contained both B_1 and B_2. The condition of all the ninetyone candies were good and they were not mouldy at all. So obviously the incidence of the toxin in the candies must be mainly due to the inclusion of spoiled groundnuts during their manufacture. Spoiled and mould damaged nuts are usually bitter to taste. Normally when people happen to eat such nuts, they spit it out, because of their highly disagreeable taste. But, as the groundnut candy contains sugar or jaggery (brown suga), the taste of the candy will not reveal the presence of the mould and so the candies will be eaten without knowledge by the children as well as adults, creating a possibility of ingestion of aflatoxin through this medium. Thus, it becomes very obvious that the prevention of contamination can be done only at the manu-

facturer's level by proper selection of good quality groundnuts, and also by thorough picking of damaged and rotten nuts manually. The nuts are liable to spoil easily under adverse conditions.

Groundnut is a highly susceptible medium for the growth of *A. flavus* and *A. parasiticus* and for the elaboration of aflatoxins. As already discussed, post-harvest drying and handling of this commodity requires careful attention by the producers. The levels of fungal contamination and the levels of toxin were found to be invariably higher in the *Kharif* season by Rao *et al.* (115). Another survey of kernels drawn from Gujarat, Andhra and Tamil Nadu States showed that 20-40% of the kernels contained aflatoxin at high levels (116). During a survey in 1967-68, nearly 50% of the 500 samples of groundnuts from west coast was found to contain aflatoxin at fairly high levels by Wagle (125). In another survey conducted at Mysore, out of 85 samples of groundnut kernels analysed 79 were found positive for the presence of aflatoxin, and the quantity of the toxin ranged between 0-100 μg/kg (106).

Similar results have been obtained by various workers in several other countries and many nations have set up maximum permissible limits for this toxin in foods and feeds. Again we would like to stress the point that some Governmental monitoring and regulatory agency should be set up in our country also.

(ii) Pickles :

In this survey, lime pickles were purchased from different markets, mainly in the form of single serve unit packages, either in polythene pouches or in pouches made of dried leaves. These are usually prepared with salt and spices and they may also contain a little oil. Generally, when there is enough mould growth on the pickle, it becomes unfit for human consumption and consumers may not purchase it. The pickles are also stored and sold in glass containers by the established big manufacturers. At homes they are stored either in glass or glazed pottery jars or plastic containers. The mould growth is possible whenever there is insufficient concentration of salt or heavy initial mould contamination or the use of insufficient amounts of oil. In such cases, when there is mould growth, it is a common practice in our country to remove the surface mat of the mould and consume the pickle. Many times people also keep the spoiled pickles under the sun for a few hours before consumption. In the present study, 37 samples of pickle (mostly lime) were analysed and six of them were found to be contaminated with aflatoxin. While three of these samples were found to be visibly affected by mould growth, three others were in generally good and acceptable condition. Out of these, two showed the presence of B_1 and B_2 while the remaining four had B_1 only. It appears that pickles also could be a very serious source of contamination in our dietary, unless proper precautions are observed.

Lime pickles have high titrable acidity and low pH. We are not sure whether aflatoxin production is possible under such conditions. Shih and Marth (126) found that presence of 1-3% sodium chloride enhanced both aflatoxin production and release of the toxin from the mycelium when compared to results with salt either absent from the medium or present at concentrations above 3%. There are reports of experimental production of aflatoxin in various fruits like apple, apricot, grape, grape fruit, orange, peach, pear and pineapple. Thus it appears that acidity also is no bar for the production of aflatoxin in fresh produce. It is obvious that there is an urgent need in our country to educate people in avoiding the use of mould infected foods in general.

(iii) Dried Vegetables :

In South India, certain vegetables like *Sundaikkai (Solanum torvum)*, cluster beans *(Cyamopsis tetragonoloa)*, chillies *(Capsicum frutescens)*, okra *(Abelmoschus esculentus)*, *manathakkali (Solanum nigrum)*, bitter gourd *(Momordica Charantia)*, *kovaikkai (Coccinia cordifolia)*, brinjal *(Solanum melongena)* field beans *(Dolichos lablab)*, *avaraikkai (Vicia faba)* etc. are commonly dried at homes, under the sun. Usually, the preparation of these consists of blanching either in steam or in boiling water,

salting and/or immersing in buttermilk for a short period, draining and drying. Once properly dried, they keep for a very long time except for discoloration. These are used in diets as side dishes after frying or in some gravies or sauces like *Vathakulambu*. It is also a practice to powder them and mix with rice for consumption.

Our study included 43 samples of such products, out of which four exhibited contamination with aflatoxin. The general condition of all these contaminated samples were found good and there was no visible mould growth. While three showed the presence of B_1, one was positive for both B_1 and B_2 also. All these four samples were dried *Sundaikkai*. Usually such *vathals* (dried vegetables) are prepared during sunny days and the drying is normally complete within 2 to 3 days. However, when the sky is overcast or whenever there are unexpected rains, the product may take more than a week or so for safe drying. Further, while sun-drying, the materials get exposed to environmental dust, flies etc. During these periods, mould contamination can occur with the concomitant production of mycotoxins. So we feel that the incidence of aflatoxin in such products may be mainly during the drying periods and not after they are dried and stored. Hygienic methods of preparation and quicker drying are also important. Once the product is dry, there may be enough salt concentration to inhibit further mould growth.

(iv) Dried Dates

Dried dates are generally imported from West Asian countries. They are commonly sold in all parts of Tamil Nadu either packed in polythene bags or loosely without any package. These are sold in shops as well as in push carts by the vendors. Generally this commodity is marketed under very unhygienic conditions with plenty of access to house flies, wherever they are sold. During rainy seasons, when the relative humidity is high, the product absorbs moisture and it is also possible for mould growth to develop. However, we understand that the trade in this commodity is highly unhygienic even from the place of its origin, *i.e.* West Asian countries. It appears that no standards are followed. We understand that even the attractively well packed dates originate from the same ship load of materials and the packing is done in the importing countries only. Eighteen samples of dates were collected from three districts (South Arcot, The Nilgiris and Thirunelveli). Except for one, others were normal looking. The lone sample (2344) which was found to be insect damaged and which also had white patches was found to be contaminated with aflatoxin B_1. The previous history of this sample is not known. Since dates are purchased commonly by people from all walks of life and since it is found to be a possible source of contamination, proper monitoring of this commodity is essential. Quality control steps have to be taken at all stages of its production, transporation and marketing. Further work in this line is warranted.

(v) Scented *Supari*, Arecanut and its Flakes *(Seeval)*

Pan chewing is a common habit amongst almost all sections of our society. *Pan* invariably consists of betel leaves, arecanut *(Areca catechu)* in its various forms and lime paste *chunnam*, with other optional ingredients like tabacco, condiments, etc Supari is sold in consumer size packets and also sold loosely in retail. Arecanut is further processed to obtain flakes and also different varieties of supari. Both arecanuts and supari are capable of becoming mouldy during various stages of their production, storage and marketing.

A total number of 147 samples were collected from different areas of the State and analysed. These consisted of scented supari (67 samples), arecanut (54 samples) and arecanut flakes (26 samples). Two samples of supari and one arecanut sample were found to contain aflatoxins. The contaminated samples of supari did not have visible mould growth. One contained both B_1 and B_2 and the others contained only B_1. The arecanut sample was found to be contaminated with visible mould growth and it had only aflatoxin B_1. The implications of using such contaminated nuts as a health hazard of human population has already been discussed. Possibly this area requires further elaborate investigations and suitable control measures.

(vi) Fresh Produce

Thirtysix samples of fresh produce was purchased for analysis from Coimbatore market only. This included grapes (3 samples), banana (5 samples), lime (1 sample), tomato (7 samples), papaya (9 samples), orange (3 samples), gooseberry (1 sample), apple (6 samples) and custard apple (1 sample). Survey work with respect to these commodities was not thorough, as we could not include sufficient number of samples under each item. These samples were generally about to rot and they were not premium grade samples. One sample of apple showed the presence of aflatoxin B_1. Generally, such items are priced low, and are purchased by people of lower economic status. It is also essential to survey various vegetables for contamination. Many times the lower grades in these items are purchased by restaurants and hotels as they are much cheaper. Contamination of fresh produce with aflatoxins has already been referred to earlier under pickles. A detailed discussion under this head has not been attempted because of lack of sufficient number of samples. However, further work in this area would prove more beneficial.

(vii) Dried Coconut

During our studies conducted in this department on the preservation of broken coconuts by salt penetration and drying, it was found that a few coconuts developed mould growth. The coconuts were found to be attacked by a large number of different mould species. Six such samples were included in our analysis for mycotoxins. Of these, two were found to be contaminated with aflatoxin B_1. Thus we find that coconut also could be a good medium for the production of such toxins, when mould growth occurs on them.

Coconut is a commonly used condiment in the South Indian cookery and is used with relish by all sections of population. It is also used in the form of chutney in houses, hotels and restaurants. Once the shell of the coconut is broken, it looses the protection, and mould growth commonly occurs in the halves within one or two days. It is a common practice at homes to leave the unspent portions in the shell for later use and keep them either in salt or as such. During such occasions, mould growth invariably occurs either mildly or severely. It is a common practice followed by the housewives, to scrape off the top mouldy layers and use the bottom portions for consumption. The occurrence of aflatoxin has been reported in copra earlier (119). Coconut oil is pressed after drying the coconut. As discussed under coconut oilcake, 4 out of 10 samples of coconut oil were found contaminated with aflatoxin (106). This clearly indicates that coconut also is a good substrate for the production of aflatoxin under suitable environmental conditions. It is felt that work in this area requires careful further consideration because of its frequent use in day-to-day cookery.

Foods Negative For Mycotoxins

The survey work included a large number of various other foods also. They were dhals and legumes and other prepared food products like candies made from roasted bengal gram and sesame seed, *kamarkat* (a low priced candy), *papads,* pounded rice, bakery products like bread, bun, cakes, biscuits, *varki, vermicelli,* dried grapes, fruit products like canned jams etc.

These items are quick selling items and are not usually stored for long time. We did not come across mouldy samples of these items during our survey, and they were not found to contain any of the mycotoxins under reference. However it is not uncommon to see some of these items becoming mouldy in our hou s s during storage. The possibility for these items to contain different mycotoxins are there and further survey work in such items will be very useful.

Microbiological Analysis

Out of the 2712 samples collected, only 1016 samples could be microbiologically analysed. The research assistant in microbiology resigned from the scheme and a substitute was not posted till the end of the project period. Hence, the microbiological assay was not completed. Even for the analysed samples, detailed identification of all the moulds could not be undertaken for want of an experienced mycologist.

The microbiological work was done mainly with respect to foodgrains and a few samples of feed during the time, when the microbiological assistant was in duty. The details of fungal population found in these samples are given in Table No. 11 at the end of the report.

It was seen that most of the samples were found to be contaminated with storage fungi rather then field fungi. *A. flavus, A. niger, Mucor, Rhizopus and Penicillium* species. were commonly encountered. Apart from these, various other fungal populations were present which could not be identified. With respect to the suspected poultry feed samples (Nos. 998 to 1016), the samples were found to be highly contaminated with *A. flavus* as the principal mould contaminant. The mould spore count indicated that the load ranged from $13x10^3$ to $142x10^3$ spores/g of the sample. Apart from these a major emphasis on microbiological analysis could not be given during our studies.

Development of Spray Reagents for the Detection of Penicillic Acid on TLC Plates

During our preliminary screening work on the survey of different foodgrains for various mycotoxins, we experienced considerable difficulty in visualizing penicillic acid by the already developed procedures like conversion of penicillic acid by ammonia fumes to a blue fluorescent spot on thin layer chromatographic (TLC) plate (40). Similarly, penicillic acid is reported to give a green spot under visible light and blue spot under long wave UV light on treatment with *p*-anisaldehyde In our studies, many blue spots and streaks were seen in the crude extracts. Further, the intensity of colours developed with *p*-anisaldehyde was poor. So, it became necessary to develop alternate procedures specific for penicillic acid. We have found that *p*-tolualdehyde and *p*-dimethyl aminobenzaldehyde give brightly coloured spots with penicillic acid under visible and longwave UV light. The procedures and usefulness of this method was reported earlier by us (72).

With TA, the spots appeared Phlox purple 632/2 (73) under visible and under UV light. The colour was stable for 15 mins. and then it slowly changed towards yellow and faded off from visible light within 6 hours. But under UV light although the colour slowly faded, it was noticeable for 24 hours. Different concentrations of penicillic acid were tested and quantities as low as 1.0 μg could be detected and 2.5 μg gave bright spots.

With DABA, the colour developed was chrome yellow 605/2 under visible light and primrose yellow 601/1 (73) under long wave UV light. Quantities as low as 0.25 μg of penicillic acid could be detected while 1.25 μg gave a bright spot. The colour was stable for 30 mins. after which it started turning brown, and in 7 hours the colour faded considerably both in visible and UV light.

With both the spray reagents the colour could again be visualised by reheating the plates briefly at 130° C. The colour of the spots intensified with an increase in the concentration of the toxin. So also the colour inten ified with an increase in the concentration of the aldehyde in both the spray reagents and we found that 1.0 ml (TA) or 1.0 gm. (DABA) of the aldehyde gave the best result. The above results were verified using spiked samples of wheat and rice to which known amounts of penicillic acid were added. These two new spray reagents were found useful in our later analysis for this toxin as confirmatory test.

Summary

During the period of three years, a total number of 2,712 samples were collected from different parts of Tamil Nadu and analysed. These included various foodgrains, food products, fresh fruits and vegetables, oilseed cakes and animal feeds. The samples were chemically scanned for the possible presence of various mycotoxins. Mycological analysis was also carried out in respect of 1016 samples for the identification of various mould species present on them.

On chemical analysis, 112 numbers of the collected samples (4.13 per cent of the total number) were found to be contaminatad with any one of the mycotoxins under reference.

Sterigmatocystin in Grain Samples

A total number of 940 samples of foodgrains which included wheat, rice and millets were analysed. All these were procured mainly from the godowns of government warehousing agencies like Food Corporation of india, Tamil Nadu Civil Supplies Corporation, Central Warehousing Corporation etc. The rice included both raw and parboiled types and wheat included domestic and imported red and white wheats. The presenco of sterigmatocystin was detected in twenty samples of rice and wheat. Most of the samples which were positive for sterigmatocystin were highly mould infected, discoloured and caked up. Such grains are unfit for human consumption. Even a few normal looking samples showed the presence of this toxin. Millions of tonnes of foodgrains are procured by the various warehousing agencies throughout our country at various times of harvest at different moisture levels. These are all sacked and stacked in various warehouses for months together for release to the public at a later date depending upon the need. From our experience, we find that the warehousing staff focus their attention mainly on the control of quantitative losses from insects and rodents. Since the loss through fungal growth is qualitative and not explicit, enough attention is not paid or no attention is paid to the damage caused by the fungi. We feel that it is highly essential that an awareness relating to fungal problems, especialiy, chronic health hazards induced by mycotoxins in the human system, is created among the warehousing staff. The procurement agencies should also be made aware of these problems and provision should be made for not purchasing foodgrains of high moisture content.

We have found sterigmatocystin in cereal grains, which is also equally toxic like aflatoxin. Our studies clearly indicate that there is a need for educating the procurement agencies as well as the warehousing staff in respect of fungal contamination and mycotoxin hazards. Scientific methods of storage are not being practised in most of the rural as well as urban warehouses. This along with the poor quality of storage structures result in gross mould infestation of the stored grains, legumes and oilseeds. Generally, the *Aspergilli, Fusaria* and the *Penicillium* are the predominant groups of moulds found on stored crops in our country. As a result of such fungal invasions, spoilage of foods occurs along with contamination by mycotoxins. So, evolving scientific storage structures which are economically viable, should be undertaken by different scientific organizations.

Sterigmatocystin in Cotton Seed

A total of thirty samples of cotton seed were purchased from the market for the survey. Sterigmatocystin was detected in two samples. The general condition of both these samples was found good.

Cotton seed is generally used for oil extraction and after refining, the oil is largely used by the *vanaspathy* manufacturing industry. The cake is used for making compounded animal feeds. Cotton seed sold in the markets are mainly meant for dairy cattle. Dairy farmers, whether big or small, purchase the same for increasing the milk yield. Usually it is difficult to recognise mould growth on cotton seeds and unless the sample is highly discoloured and gives bad odour, people

do not hesitate to purchase the material. Since sterigmatocystin is a toxin closely related to aflatoxin, it is capable of causing severe damage to the vital organs of the cattle and also poses a severe health hazard to the human beings through the secretion of toxic metabolites in milk. Again, this study highlights the importance of proper post-harvest storage and handling of this commodity so that fungal invasion and concomitant toxin production is minimised.

Patulin in Scented *Supari*

Supari (processed arecanut, *Areca catechu)* is chewed along with *pan* (betel leaves) by vast sections of our population, poor as well as rich, and by people of both urban and rural areas. Sixtyseven samples of scented *supari* packed in wax paper in unit sized consumer packets of 2-3 gms. were purchased and analysed. One sample was found to contain patulin.

The contents of these packets could be seen caked up with diffuse mould growth on them. This can occur during storage under adverse humid conditions. Arecanut also is susceptible for spoilage due to fungal invasion during its post-harvest handling. We feel that mycotoxin development is mainly from the original spoiled and mouldy raw material. Since various flavourings and colourings are used in the manufacture of scented *supari,* any bad smell or change in colour or appearance due to contamination gets highly masked. So it will be very difficult to detect damage after processing unless fresh mould growth occurs. Unscrupulous utilisation of mouldy arecanuts in the manufacture of scented *supari* might cause the toxin to get diffused over a large number of packets as the nuts are further broken down into small particles. So it is very much essential that care has to be exercised in the post-harvest operations like de-husking drying and handling. People should be educated in rejecting the consumption of mouldy arecanuts and scented *supari* packets. Similarly processors also should be informed of the danger of including damaged nuts in the manufacture of *supari* packets.

Penicillic Acid in Apples

Six samples of apples purchased in the local market were analysed. In one sample, where the rot had set in and patches of green mould growth had started, penicillic acid was detected to be present.

In our country such apples are graded low with substantial reduction in prices and they are purchased by the economically weaker sections of the society. Rotting and moulding of fruits generally start with bruising on their surface during harvesting and transportation. Further, apples are cold stored and when they are taken out for marketing moisture will condense over their surface creating a congenial atmosphere for moulds to develop. Refrigerated transportation or refrigerated display facilities are not available in our country. The produce requires gentle and careful handling during its entire post-harvest stages of storage, transport and retail marketing. Atleast physical damages and bruises should be avoided since bruising exposes the cell sap for rapid microbial decay. So, precautionary measures like gentle handling and proper packaging have to be practised by wholesalers and retailers as well. Besides the severe economic loss due to low grading and rejection, the produce becomes susceptible for mycotoxin contamination.

Aflatoxin in Various Commodities

Aflatoxin contamination was detected in a wide variety of commodities including foods and feeds.

Cotton Seed :

Two samples of cotton seed out of thirty analysed were found to be positive for aflatoxin. They contained both aflatoxin B_1 and B_2, and the samples were found to be discoloured on inspection.

Cotton seed and its cake is used largely as dairy cattle feed. Its protein concentrate is also considered for large scale supplementation of human diet in various parts of the world. Ingestion of aflatoxin by the farm animals causes aflatoxicosis in them. Besides affecting their health, milk

production is greatly reduced. In severe and acute cases, they cause mortality of dairy cows resulting in serious economic losses. Further, aflatoxins are secreted in the milk in the form of their derivatives aflatoxin M_1 and M_2, which may lead to severe public health problems. This study emphasises the importance of proper post-harvest drying and storage of cotton seed to avoid mould growth.

Groundnut Oilcake

It has been well recognised that aflatoxin is a serious contaminant in groundnut oilcake. Out of 134 samples analysed, twentyeight samples recorded positive presence of aflatoxin. Fifteen of the samples contained both aflatoxin B_1 and B_2 while the rest of the positive samples contained only B_1.

Moulds of the *Aspergillus* species especially *A. flavus* and *A. parasiticus,* grow on groundnut when they are stored after harvest without adequate drying. These secrete the aflatoxins on groundnut and other commodities. During harvest seasons, when there is high moisture in the crop, the atmosphere is highly humid and the crop is severely susceptible to mould growth and toxin contamination. Since the toxin gets into the oil while pressing and since groundnut oil is a popular edible oil with our people, aflatoxin is a serious animal and human health hazard in our country. Proper drying of the crop and well ventilated storage are highly necessary.

Gingelly Oilcake

This study has shown that even gingelly oilcake could be a good substrate for the production of aflatoxin. Eightythree samples of this commodity were analysed and four were positive for aflatoxin. Three of them contained both aflatoxins B_1 and B_2 and one of them contained B_1 only. Thus, it is found proper post-harvest drying and storage of gingelly oilseed is a must to avoid mycotoxin contamination in the oil and cake.

Coconut Oilcake

Ninetysix samples purchased from different markets were analysed and three of them were found to be contaminated with aflatoxin B_1. Coconut oilcakes are often used in chutney preparations in hotels either as a substitute or as an adulterant by unscrupulous elements. So there is a good chance of this item getting indirectly into the human dietary. Occurrence of heavy mould growth during drying of the broken coconuts, before oil extraction, is a common sight in coastal areas. Coconut oil is also widely used in places of production and the toxin gets distributed between the oil and cake during the oil pressing. This study stresses the importance of precautionary measures that should be taken for the prevention of mould growth during drying of coconut halves.

Feed Concentrates and Oilseed Cake Mixtures

Feed concentrates have become very popular recently under different trade names. Thirtyeight samples purchased from markets were analysed and it was found that seven of them were contaminated with aflatoxins. Of these, five contained B_1 only while two of them contained aflatoxins B_1 and B_2 as well. These feeds are compounded from various ingredients like groundnut oilcake, maize, sorghum, fish meal, etc. purchased from various sources and unless strict quality control is practised for each commodity, severe economic loss due to decrease in milk production, illness and even death of the animals will result through the use of contaminated feeds. Many feed manufacturers do not have separate quality control system, with the result the contaminated samples easily enter the market.

In certain parts of this State, the local shopkeepers blend different oilseed cakes in different proportions for animal feeds. Out of ten such samples analysed, four were found to be contaminated with aflatoxin B_1.

Poultry Feeds

Poultry feeds are manufactured in great quantities, in our country. A total number of 60 samples, both purchased and sent by different agencies, were analysed. In an area around Erode during 1977, there was a heavy drop in egg production and high mortality of birds in all the

poultry farms. It was found by us that most of the samples of feed when analysed contained a large amount of aflatoxins at a concentration 200 to 1000 μg/kg. In all the positive samples *A. flavus* was found as the principal mould contaminant. The fungal load ranged between $13x10^3$ and $145x10^3$ *A. flavus* spores per gm. of the sample. During 1978, forty more samples were analysed and two samples were positive for aflatoxin B_1.

Incidence of mycotoxicosis in poultry is widely prevalent in India. Only when there is a severe loss or damage, farmers think of getting their feeds analysed. Otherwise the losses go unnoticed. The toxin seriously affects the productivity of the birds and since the toxin is secreted in the white and yolk of the eggs, there is also a serious health hazard to human beings.

Groundnut Candies

Out of 91 samples of groundnut candies analysed, four were found to be contaminated with aflatoxin. The incidence is mainly due to the inclusion of spoiled groundnuts during the manufacture. Since the bitter taste of the spoiled groundnuts is masked by the inclusion of jaggery in the candy, children and adults will consume them unknowingly. Thus this item becomes a direct medium of ingestion of aflatoxin by humans. Groundnut is highly susceptible for contamination and unless the spoiled nuts are carefully rejected during manufacture, a serious health hazard will result.

Pickles

Lime pickles purchased in the form of single serve unit packages either in polythene pouches or dried leaf pouches were analysed. Out of 37 samples tested, six were found to be contaminated with aflatoxin. It is a common practice in our country to remove the surface mat of mould and consume the pickle. Thus pickles could become a serious source of contamination in our dietary unless proper precautions are taken to follow correct recipes and hygienic manufacturing practices to prevent mould attack.

Dried Vegetables

Our study included 43 samples of dried vegetables or *vathals* of different kinds purchased at various places. Four samples of *Sundaikkai (Solanum torvum)* exhibited contamination with aflatoxin. One of these was positive for both aflatoxins B_1 and B_2. Normally these vegetables are salted and dried in the sun. Usual drying time is 2 to 3 days for reaching safe moisture levels. Whenever the sky is overcast or it rains unexpectedly, the drying time gets increased and the product being still wet becomes susceptible for mould growth, and for possible mycotoxin contamination. Hygienic methods of preparation and quick drying is important in the manufacture of these products.

Dried Dates

Dried dates are very common in our State where it is sold in shops and through push carts both packaged and also in loose quantities. Generally, this commodity is handled under very unhygienic conditions with plenty of access to houseflies, insects etc. Eighteen samples were purchased and analysed. One sample which was found to be insect damaged and which also had white patches was found to be positive for aflatoxin B_1. Since dates are commonly purchased by people of all walks of life, careful monitoring of this commodity during import is essential. Hygienic standards have to be prescribed to the exporting countries and should be strictly watched at the points of entry into our country.

Scented *Supari*, Arecanut and Arecanut Flakes

A total number of 147 samples consisting of scented *supari* (67 samples), arecanut (54 samples) and its flakes (26 samples) were surveyed. Two samples of *supari* and one arecanut sample were positive for aflatoxin. One of the positive *supari* samples was found to be contaminated with both aflatoxins B_1 and B_2.

As already discussed, *pan* chewing is a common habit in India. This study emphasises the importance of educating both the public and

the processors on the health hazards of using mouldy and infected arecanuts as *supari.*

Dried Coconuts

Six samples of dried coconuts which had developed mould growth on them were analysed. Two of the samples were found to be contaminated with aflatoxin B_1. Thus it is found that coconut also could be a good medium for mycotoxin production. Coconut is a common item used in South Indian households. Once the coconut is broken into halves, mould growth invariably occurs. Housewives generally scrape off the top mouldy layers and use the bottom portions for consumption. Since the toxin penetrates into deep layers also such practice can lead to serious health hazards. There are reports that coconut oil and coconut oilcake were found to be contaminated with aflatoxin. Since no careful attention is given during drying of coconuts in the coastal areas, the contaminated nuts can enter into human dietary directly through the oil and pose dangers. Here also, educating the housewives and processors against using the contaminated coconuts is important.

Foods Negative for the Mycotoxins

The survey work included a large number of other food products also. They are dhals and legumes, candies made from roasted bengal gram and sesame seed, *kamarkat, papads,* pounded rice, bakery products like bread, bun, biscuits, *varki, vermicelli,*, dried grapes, canned jams, different brans meant for animal feeds etc. We did not come across mouldy samples of these items and they were also not found to contain any of these mycotoxins.

Development of Spray Reagents for the detection of Penicillic Acid

Two alternate spray reagents for the detection and confirmation of penicillic acid on TLC plates were developed, during the course of our survey. p-tolualdehyde and p-dimethyl amino benzaldehyde were found to give brightly coloured spots with penicillic acid under visible and long wave UV light helping the easy identification of this toxin on TLC plates.

Conclusions and Recommendations

Mould spores are generally present in the atmosphere and all foods and food products are susceptible for mould attack. Soon after harvest, foodgrains are stored without proper drying, due to lack of sunshine or rains or even ignorance. Similarly, fresh horticultural produce are also not properly handled after their harvest with the result, they get bruised providing an avenue for fungal growth. Besides, the tropical weather conditions of high humidity and relatively high temperature, present in India, favour mould growth. Proper drying facilities with necessary machineries are not available in our country and, whenever available, such processes increase the cost of the commodities. Proper packaging of the commodities is not practised and with the result the processed food products are mould contaminated. Mouldy groundnut is a serious health hazard and no concerted attempt is being taken in the oil mills to separate the spoiled nuts. This results not only in the loss of thousands of tons of good quality protein for human consumption, but also poses a serious health hazard by the introduction of aflatoxins into the human system through the use of groundnut oil and cakes in feeds through milk.

Although refining of the oils is known to destroy the mycotoxin, common people prefer unrefined crude oil, because of its cheapness and natural flavour. The study has revealed presence of aflatoxins in gingelly and coconut oilcakes and also in cotton seeds. So further efforts in the study of mycotoxins in these oils also may prove beneficial.

Various feed manufacturers use different oilseed cakes in their feeds. These contaminated feeds may introduce mycotoxins into the human system through meat, milk and eggs. The study has also revealed mycotoxin contamination in cereals like wheat and rice which are distributed through public distribution systems.

The oilseed cakes are invariably used as animal and poultry feeds. Our study has establish-

ed that these oilseed cakes do contain aflatoxin and there is severe danger to the human health, because of possible contamination of animal products like milk, meat, eggs etc. Similar results had been obtained by earlier workers also. It is recommended that the government should establish monitoring agencies, especially for detecting mycotoxins in foods and feeds. Such agencies are already operating in almost all the advanced countries.

It has been the general practice in India to regard mould infected foods as harmless. Whenever such contaminated foods are encountered, often people remove the mould growth and consume the food. This practice is most noticeable in the poorer sections of our society, who are already malnourished. The inclusion of mouldy foods containing mycotoxins in their diet should be viewed with serious concern, because of the chronic toxicity of these toxins and the low nutritional status of our people. Further, whenever the fungal growth is too severe for use as human food, the generally accepted practice in India is to use the same as animal feed. It has been established that these toxic metabolites are equally very harmful to cattle and poultry, and further, there is also the danger of contamination of the animal products like milk, meat etc.

Thus, it becomes obvious that there is a great need for disseminating this knowledge and educating the public with regard to the possible dangers of consuming mouldy foods. This should be done through popular articles, by inclusion of this topic in the school curriculum, by periodical radio talks (farmer's programmes, programmes for children and those for women), by exposition during *melas* and village fairs, and through the various extension agencies existing in the blocks *(mukhya sevikas* and extension assistants). This will create the necessary awareness about mycotoxins and their health hazards amongst the public.

Knowledge on problems created by fungal invasion and mycotoxin contamination should be imparted to the staff employed in warehousing and procurement agencies so that they will be able to appreciate this quality loss and the implied health hazard to the public.

Proper drying facilities like paved yards, and cheap solar drying machineries should be made available in the countryside for drying grains and other produce rapidly and economically to safe moisture levels.

Scientific storage structures which are economically viable and local should be evolved using appropriate technology.

Lastly, it is again emphasised that the government should establish monitoring agencies and set up laboratories for the surveillance of foods and feeds for mycotoxins.

Table 4.

Fresh Produce Samples Collected From the Local Coimbatore Market

Type	No. of Samples
Grapes	3
Banana	5
Lime	1
Tomato	7
Papaya	9
Orange	3
Gooseberry	1
Apple	6
Cluster Apple	1
Total	36

Table No. 5

Types of Samples Collected — Cereals and Pulses

Serial No.	Place	District	Rice	Wheat	Sorghum	Bajra	Samai	Maize	Milo	Red gram	Black gram	Green gram	Bengal gram	Toor dhal	Bengalgram dhal	Urad dhal	Greengram dhal	Pea dhal	Mixed dhal	Cow pea	Pea	Beans	Horse gram	Total
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1.	Coimbatore TNCSC, FCI and local market	Coimbatore	146	122	7	1	-	-	1	12	10	2	-	8	7	8	-	-	-	1	1	3	-	329
2.	Tiruppur	,,	5	1	3	-	2	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	12
3.	Somanur	,,	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
4.	Pollachi	,,	5	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9
5.	Udumalpet	,,	3	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5
6.	Vadamadurai	,,	-	-	-	-	-	-	-	-	-	3	-	3	5	6	-	-	-	-	1	-	-	18
7.	Mettupalayam	,,	2	1	-	-	-	-	-	-	2	1	1	5	6	3	-	-	-	2	1	2	1	27
8.	Perur	,,	-	-	-	-	-	-	-	-	-	1	-	2	2	2	-	-	-	-	-	-	1	8
9.	Sulur	,,	10	5	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	16
10.	Peelamedu	,,	10	9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	19
11.	Singanallur	,,	8	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	24
12.	Erode	Periyar	3	1	-	-	-	-	-	-	-	5	-	12	10	10	3	4	1	2	2	6	1	60
13.	Perundurai	,,	-	-	-	-	-	-	-	-	-	-	-	1	1	1	-	1	1	-	-	-	-	5
14.	Bhavani	,,	10	3	1	-	-	-	-	-	-	-	-	5	5	5	5	2	-	-	-	-	-	36

Table No. 5 continued

Serial No.	Place	District	Rice	Wheat	Sorghum	Bajra	Samai	Maize	Milo	Red gram	Black gram	Green gram	Bengal gram	Toor dhal	Bengalgram dhal	Urad dhal	Greengram dhal	Pea dhal	Mixed dhal	Cow pea	Pea	Beans	Horse gram	Total
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
15.	Ooty	The Nilgiris	-	-	-	-	-	-	-	-	-	-	-	10	10	7	-	-	-	6	1	16	2	52
16.	Karur	Trichy	-	-	-	-	-	-	-	-	-	1	-	10	4	5	-	-	-	1	-	-	-	21
17.	Kulithalai	,,	-	-	-	-	-	-	-	-	-	1	-	1	1	2	-	-	-	-	-	-	-	5
18.	Pettavaithalai	,,	-	-	-	-	-	-	-	-	-	1	-	3	3	1	-	-	-	-	1	-	-	9
19.	Trichurapalli	,,	50	97	-	-	-	-	-	3	3	2	-	5	4	1	-	-	-	-	3	-	-	168
20.	Thanjavur	Thanjavur	4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4
21.	Vallam Airstrip	,,	6	20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	26
22.	Mannargudi	,,	5	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10
23.	Thirunellikkaval	,,	-	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11
24.	Sembonnarkoil	,,	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10
25.	Nagappattinam	,,	6	17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	23
26.	Manalur	South Arcot	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10
27.	Chidambaram	,,	2	-	-	-	-	-	-	-	-	1	4	3	3	5	3	-	-	-	2	1	1	25
28.	Cuddalore	,,	3	-	-	-	-	-	-	-	-	-	1	4	5	3	3	-	-	-	-	-	-	19
29.	Sirkali	Thanjavur	-	-	-	-	-	-	-	-	-	4	4	4	3	4	3	-	-	-	1	1	1	25
30.	Mayuram	,,	-	-	-	-	-	-	-	-	-	-	4	4	5	5	3	-	-	-	5	-	-	26
31.	Melanikkuli	Trichy	-	-	-	-	-	-	-	-	-	1	1	1	4	3	1	-	1	-	1	1	1	15

Table No. 5 continued

Serial No.	Place	District	Rice	Wheat	Sorghum	Bajra	Samai	Maize	Milo	Red gram	Black gram	Green gram	Bengal gram	Toor dhal	Bengalgram dhal	Urad dhal	Greengram dhal	Pea dhal	Mixed dhal	Cow pea	Pea	Beans	Horse gram	Total
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
32.	Ariyalur	Trichy	-	-	-	-	-	-	-	-	-	2	3	3	5	2	-	-	-	1	1	2	-	19
33.	Arakkonam	North Arcot	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12
34.	Egmore	Madras (city)	30	35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	65
35.	Avadi	,,	17	85	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	103
36.	Sholavaram	,,	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10
37.	Madras	,,	-	-	-	-	-	-	-	-	-	9	-	24	25	21	-	-	-	2	4	-	2	87
38.	Theni	Madurai	-	5	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	6
39.	Dindigul	,,	1	4	-	-	-	-	-	-	-	-	-	4	3	1	-	-	-	-	-	-	-	13
40.	Madurai	,,	1	13	-	-	-	-	-	-	-	1	-	3	3	1	-	-	-	-	-	-	-	22
41.	Aruppukkottai	Ramanathapuram	-	-	-	-	-	-	-	-	-	-	-	2	1	2	-	-	-	-	-	-	-	5
42.	Sivagangai	Madurai	-	-	-	-	-	-	-	-	-	-	-	1	2	1	-	-	-	-	-	-	-	4
43.	Manamadurai	,,	-	-	-	-	-	-	-	-	-	-	-	2	2	2	-	-	-	-	-	-	-	6
44.	Tirupuvanam	,,	-	-	-	-	-	-	-	-	-	-	-	2	2	2	-	-	-	-	-	-	-	6
45.	Virudhunagar	Ramanathapuram	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12
46.	Tuticorin	Tirunelveli	-	28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	28
47.	Pondicherry	Pondicherry	7	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	17
48.	Karaikkal	,,	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5
	Total		360	528	12	1	2	1	1	16	17	33	17	122	119	106	23	7	3	15	24	32	10	1449

Table No. 6 continued

Sl. No.	Place	District	Rava	Maida	Vermicelli	Biscuits	Varki	Bread, Bun, Cake	Pounded Rice	Papad	Pickle	Groundnut candy	G ingelly candy	Kammarkat candy	Roasted Bengal gram candy
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
26.	Madurai	Madurai	-	-	-	-	-	-	-	-	-	-	-	-	-
27.	Aruppukkottai	Ramanad	-	-	-	-	-	-	-	-	-	-	-	-	-
28.	Sivagangai	Madurai	-	-	-	-	-	-	-	-	-	-	-	-	-
29.	Dindigul	,,	-	-	-	-	-	-	-	-	-	-	-	-	-
30.	Manamadurai	,,	-	-	-	-	-	-	-	-	-	-	-	-	-
31.	Thirupuvanam	,,	-	-	-	-	-	-	-	-	-	-	-	-	-
32.	Thirupparangundram	,,	-	-	-	-	-	-	-	-	-	-	-	-	-
33.	Vadamadurai	Coimbatore	-	-	-	-	-	-	-	-	-	-	-	-	-
34.	Perundurai	Periyar	-	-	-	-	-	-	-	-	-	-	-	-	-
35.	Ariyalur	South Arcot	-	-	-	-	-	-	-	-	-	-	-	-	-
36.	Cuddalore	,,	-	-	-	-	-	-	-	-	-	-	-	-	-
37.	Mayuram	Thanjavur	-	-	-	-	-	-	-	-	-	-	-	-	-
38.	Tuticorin	Tirunelveli	-	-	-	-	-	-	1	3	4	2	-	-	-
39.	Nagercoil	Kanyakumari	-	-	-	-	-	-	-	1	1	7	-	-	-
40.	Tirunelveli	Tirunelveli	-	-	-	-	-	-	2	3	-	3	-	-	-
41.	Kanyakumari	Kanyakumari	-	-	-	-	-	1	2	2	1	3	-	-	-
42.	Kailasapuram	Tirunelveli	-	-	-	-	-	-	-	-	-	3	-	-	-
43.	Valliyur	,,	-	-	-	-	-	-	1	3	-	2	-	-	-
44.	Nazareth	,,	-	-	-	-	-	-	-	2	1	3	-	-	-
45.	Sawyerpuram	,,	-	-	-	-	-	-	1	3	3	3	-	-	-
46.	Avanashi	Coimbatore	-	-	-	-	-	-	-	2	-	-	-	2	-
47.	Tiruppur	,,	-	-	-	1	1	1	-	4	1	1	-	-	1
48.	Palladam	,,	-	-	-	3	1	2	-	-	-	1	-	1	1
49.	Sulur	,,	-	-	-	-	-	-	-	5	3	3	3	4	1
50.	Somanur	,,	-	-	-	-	-	-	-	3	-	2	1	2	-
51.	Karumathampatti	,,	-	-	-	-	-	-	-	1	2	1	3	-	1
52.	Feed Manufacturers	Madras city	-	-	-	-	-	-	-	-	-	-	-	-	-
	Total		7	10	47	11	5	15	12	130	37	91	9	9	(

Dried vegetables	Arecanut	Arecanut flakes	Scented Supari packet	Dried Grapes (Raisins)	Dried Dates	Miscellaneous Food Products	Groundnut	Dried Coconut	Jam	Apple Juice	Groundnut oilcake	Gingelly oilcake	Coconut oilcake	Cottonseed ilcake	Cottonseed and husk	Cattle feed	Poultry feed	Bran	Oilseed cake mixture	Total
17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37
-	-	-	-	-	-	-	-	-	-	-	7	6	2	-	-	-	1	-	-	16
-	-	-	-	-	-	-	-	-	-	-	1	1	1	-	-	-	-	1	-	
-	-	-	-	-	-	-	-	-	-	-	3	2	-	-	-	-	-	-	-	5
-	-	-	-	-	-	-	-	-	-	-	3	4	2	-	-	-	-	-	-	9
-	-	-	-	-	-	-	-	-	-	-	2	2	-	-	-	-	-	1	-	5
-	-	-	-	-	-	-	-	-	-	-	2	2	3	-	-	-	-	3	-	10
-	-	-	-	-	-	-	-	-	-	-	1	-	2	-	-	-	-	5	-	
-	-	-	-	-	-	-	-	-	-	-	7	2	2	-	-	1	1	26	-	39
-	-	-	-	-	-	-	-	-	-	-	4	-	2	-	-	-	-	3	-	9
-	-	-	-	-	-	-	-	-	-	-	3	2	-	-	1	-	2	1	-	9
-	-	-	-	-	-	-	-	-	-	-	3	2	-	-	2	-	-	1	1	9
-	-	-	-	-	-	-	-	-	-	-	4	2	-	-	1	-	-	2	-	9
-	3	-	-	-	-	-	-	-	-	-	3	-	1	-	-	-	-	-	2	19
-	1	-	-	-	-	-	-	-	-	-	3	1	-	-	-	-	-	-	1	15
-	3	-	-	1	1	-	-	-	-	-	2	-	3	-	-	-	1	-	2	21
-	3	-	-	-	-	-	-	-	-	-	1	2	-	-	-	-	-	-	1	16
1	1	-	-	1	-	-	-	-	-	-	-	2	-	-	-	-	-	-	2	10
-	2	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	1	11
-	2	-	-	2	-	-	-	-	-	-	3	1	-	1	-	-	-	-	1	16
-	2	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	4	18
-	4	-	-	-	-	-	-	-	-	-	2	-	6	2	2	-	-	-	-	20
3	3	-	-	-	-	2	-	-	-	-	2	-	3	-	2	-	-	3	-	28
-	1	-	-	-	-	1	-	-	-	-	3	1	-	-	4	-	-	-	-	19
-	-	-	-	-	-	4	-	-	-	-	5	-	5	-	-	-	-	-	-	33
4	-	-	-	-	-	5	-	-	-	-	6	-	2	-	-	-	-	4	-	29
-	-	-	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	-	10
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	19	-	-	23
3	43	54	26	67	52	18	18	3	6	3	1	134	83	96	4	30	38	60	87	15	1227

Table No. 9

Mycotoxin Positive Samples — Their type, source, condition and toxin identified

Sl. No.	Sample No.	Type of Sample	Place and Date of Collection		Condition of the Sample	Toxin Identified	Remarks
1	2	3	4		5	6	7
1.	363	Red Wheat	FCI, Virudhunagar	10-3-77	Highly infested and unfit for consumption	Sterigmatocystin	
2.	364	,,	,, ,,	,,	,,	,,	
3.	451	Raw Rice	Ration shop, Coimbatore	21-4-77	Slightly mould infested	,,	
4.	452	Rice	FCI, Egmore	24-5-77	Damaged	,,	
5.	459	Raw Rice	,, ,,	24-5-77	Normal	,,	From Punjab
6.	500	Red Wheat	,, ,,	24-5-77	Discoloured grains	,,	Imported
7.	505	Red Wheat	,, ,,	24-5-77	Normal	,,	Imported
8.	525	Raw Rice	FCI, Avadi	25-5-77	Normal	,,	
9	547	Red Wheat	,, ,,	25-5-77	Caked and black	,,	
10.	563	,,	,, ,,	25-5-77	Infested	,,	
11.	580	,,	,, ,,	25-5-77	Black, infested	,,	
12.	582	,,	,, ,,	25-5-77	Black, infested	,,	
13.	627	Wheat	FCI, Sholavaram Airstrip	27-5-77	Highly mouldy, discoloured and caked up	,,	Open stack cap storage
14.	628	,,	,, ,,	27-5-77	,,	,,	,,
15.	629	,,	,, ,,	27-5-77	,,	,,	,,

Table No. 9 continued

Sl. No.	Sample No.	Type of Sample	Place and Date of Collection		Condition of the Sample	Toxin Identified	Remarks
1	2	3	4		5	6	7
16.	630	Wheat	FCI, Sholavaram Airstrip	27-5-77	Highly mouldy, discoloured and caked up	Sterigmatocystin	Open stack cap storage
17.	666	White Wheat	FCI, Singanallur	5-8-77	Infested, damaged and discoloured	"	"
18.	752	Raw Rice	CWHC, Trichy	14-8-77	Rain affected and caked up	"	"
19.	761	Boiled Rice	TNCSC, Trichy	14-8-77	Mixed with black and caked grains	"	"
20.	794	White Wheat	CWHC, Trichy	14-8-77	Kept in open stock, black, mouldy and caked	"	Imported
21.	998	Poultry Feed	Private Poultry Farm, Erode	16-12-77	Normal looking	Aflatoxin B_1	High mortality rate and lowered egg production
22.	999	"	" "	16-12-77	"	Aflatoxin B_1 (120 μg/kg)	"
23.	1000	"	" "	16-12-77	Good	" B_1&B_2 (1000 μg/kg)	"
24.	1001	"	Kattupalayam through M.V.C. Madras	23-12-77	"	" B_1 & B_2 (500 μg/kg)	"
25.	1002	"	M. V. C. Madras	"	"	" B_1 (250 μg/kg)	"
26.	1004	"	" "	"	"	" B_1 (Trace)	"
27.	1005	"	" "	"	"	" B_1 (250 μg/kg)	"
28.	1006	"	" "	"	"	" B_1 (Trace)	"
29.	1007	"	" "	"	"	" B_1&B_2 (1000 μg/kg)	"
30.	1008	"	" "	"	"	" B_1&B_2 (1000 μg/kg)	"
31.	1009	"	" "	"	"	" B_1 (Trace)	"
32.	1011	"	" "	"	"	" B_1 (1000 μg/kg)	"

FCI — Food Corporation of India.
CWHC — Central Warehousing Corporation.
TNCSC — Tamil Nadu Civil Supplies Corporation.
M.V.C. — Madras Veterinary College, Madras.

Table No. 9 continued

Sl. No.	Sample No.	Type of Sample	Place and Date of Collection		Condition of the Sample	Toxin Identified	Remarks
1	2	3	4		5	6	7
33.	1012	Poultry Feed	M. V. C. Madras	23-12-77	Good	Aflatoxin B_1 (1000 μg/kg)	High mortality rate and lowered egg production
34.	1014	,,	,,	,,	,,	,, B_1 (1000 μg/kg)	,,
35.	1015	,,	,,	,,	,,	,, B_1 (500 μg/kg)	,,
36.	1016	,,	,,	,,	,,	,, B_1 & B_2 (1000 μg/kg)	,,
37.	1058	Cattle Feed	,,	4-2-78	,,	,, B_1 (1200 μg/kg)	
38.	1059	,,	,,	,,	,,	,, B_1 (1200 μg/kg)	
39.	1062	Poultry Feed	Poultry Farm, Erode	18-2-78	,,	,, B_1	High mortality rate & lowered egg production
40.	1431	Groundnut Oilcake	Fatimanagar	15-4-78	,,	,, B_1	,,
41.	1432	Coconut Oilcake	,,	,,	,,	,, B_1	,,
42.	1433	Gingelly Oilcake	,,	,,	,,	,, B_1	,,
43.	1434	Cattle Feed	,,	,,	,,	,, B_1	Dry coco feed mix
44.	1435	,,	,,	,,	,,	,, B_1	Milk ration without mineral mixture
45.	1436	Cattle Feed	Mannuthy	22-5-78	Appearance good	,, B_1 & B_2 (2405 μg/kg)	Through Veterinary College, Trichur
46.	1644	Groundnut Oilcake	Erode Market	10-7-78	,,	,, B_1 & B_2	
47.	1646	,,	,,	,,	,,	,, B_1	
48.	1648	Coconut Oilcake	,,	,,	,,	,, B_1	
49.	1649	Groundnut Oilcake	,,	,,	,,	,, B_1	
50.	1658	,,	Perundurai Market	,,	,,	,, B_1 & B_2	

Table No. 9 continued

Sl. No.	Sample No.	Type of Sample	Place and Date of Collection		Condition of the Sample	Toxin Identified	Remarks
1	2	3	4		5	6	7
51.	1660	Groundnut Oilcake	Perundurai Market	10-7-78	Appearance Good	Aflatoxin B_1	
52.	1680	Poultry Feed Ingredient	Coimbatore	28-8-78	,,	,, B_1	TNAU; *Enterolobium samar*
53.	1753	Gingelly Oilcake	Chidambaram Market	25-8-78	,,	,, B_1 and B_2	
54.	1754	Groundnut Oilcake	,,	,,	,,	,, B_1 and B_2	
55.	1756	,,	,,	,,	,,	,, B_1 and B_2	
56.	1758	,,	,,	,,	,,	,, B_1 and B_2	
57.	1776	,,	Melanikkuli Market	26-8-78	,,	,, B_1 and B_2	
58.	1778	,,	,,	,,	,,	,, B_1 and B_2	
59.	1779	,,	,,	,,	,,	,, B_1 and B_2	
60.	1780	,,	,,	,,	,,	,, B_1 and B_2	
61.	1808	Gingelly Oilcake	Ariyalur Market	27-8-78	,,	,, B_1 and B_2	
62.	1809	,,	,,	,,	,,	,, B_1 and B_2	
63.	1810	Croundnut Oilcake	,,	,,	,,	,, B_1 and B_2	
64.	1811	,,	,,	,,	,,	,, B_1 and B_2	
65.	1812	,,	,,	,,	,,	,, B_1 and B_2	
66.	1833	,,	Guddalore Market	28-8-78	,,	,, B_1 aad B_2	
67.	1834	,,	,,	,,	,,	,, B_1	
68.	1878	,,	Mayuram Market	29-8-78	,,	,, B_1	
69.	1883	Maize (Poultry Feed Ingredient)	Mysore Feeds Ltd., Bangalore	26-9-78	,,	,, B_1	A few grains appeared black or mouldy at the germ end
70.	1902	Dried Vegetable	Coimbatore	4-10-78	Appearance Good	Aflatoxin B_1 and B_2	*(Sundaikkai Vatral)*
71.	1950	Apple	,,	8-10-78	Towards rotting	,, B_1	

Table No. 9 continued

Sl. No.	Sample No.	Type of Sample	Place and Date of Collection		Condition of the Sample	Toxin Identified		Remarks
1	2	3	4		5	6		7
72.	1964	Scented Supari	Coimbatore	30-11-78	Appearance Good	,,	B_1	(Sundaikkai Vatral)
73.	1989	Ground Oilcake	Tuticorin	2-11-78	Appearance Good	,,	B_1	
74.	1991	Oilseed Cake Mixture	Tuticorin	2-11-78	,,	,,	B_1	
75.	2004	Pickles	,,	7-11-78	Slight Mould Attack	,,	B_1	
76.	2012	Groundnut Oilcake	Nagercoil	8-11-78	Appearance Good	,,	B_1	
77.	2013	Arecanut	,,	,,	Infected with Green Mould	,,	B_1	
78	2016	Groundnut Candy	,,	,,	Good looking	,,	B_1 and B_2	
79.	2020	Pickles	,,	,,	Mould attack (Black)	,,	B_1 and B_2	
80.	2049	Groundnut Candy	Kanyakumari	9-11-78	Good looking	,,	B_1	
81.	2056	Oilseed Cake Mixture	,,	,,	Appearance Good	,,	B_1	
82.	2058	Groundnut Oilcake	,,	,,	,,	,,	B_1	
83.	2060	Dried Vegetable	Kailasapuram	3-11-78	,,	,,	B_1	
84.	2061	Groundnut Candy	,,	,,	,,	,,	B_1 and B_2	
85.	2066	Oilseed Cake Mixture	,,	,,	,,	,,	B_1	
86.	2078	,,	Valliyur	7-11-78	,,	,,	B_1	
87.	2081	Groundnut Candy	Nazareth	3-11-78	,,	,,	B_1	
88.	2084	Pickles	,,	,,	,,	,,	B_1	
89.	2097	Pickles	Sawyerpuram	6-11-78	,,	,,	B_1	
90.	2098	Pickles	Sawyerpuram	6-11-78	With White Mould	,,	B_1 and B_2	
91.	2126	Cotton Seed	Avanashi	1-2-78	Discoloured	,,	B_1 and B_2	
92.	2129	Groundnut Oilcake	,,	,,	Appearance Good	,,	B_1 and B_2	

Table No. 9 continued

Sl. No.	Sample No.	Type of Sample	Place and Date of Collection		Condition of the Sample	Toxin Identified	Remarks
1	2	3	4		5	6	7
93.	2141	Pickles (Lime)	Tiruppur	2-12-78	Appearance Good	Aflatoxin B_1	
94.	2146	Dried Vegetable	,,	,,	,,	,, B_1	*(Sundaikkai Vatral)*
95.	2147	,,	,,	,,	,,	,, B_1	,,
96.	2176	Cotton Seed	Palladam	3-12-78	Discoloured	,, B_1 and B_2	
97.	2179	Groundnut Oilcake	,,	,,	Appearance Good	,, B_1	
98.	2208	Coconut Oilcake	Sulur	28-11-78	Mouldy	,, B_1	
99.	2209	Groundnut Oilcake	,,	,,	,,	,, B_1	
100.	2239	,,	Somanur	29-11-78	,,	,, B_1	
101.	2256	Cattle Feed	Shaw Wallace Co., Ltd., Madras	15-12-78	Appearance Good	,, B_1	
102.	2258	Copra	Coimbatore	16-12-78	Infected with Mould	,, B_1	
103.	2262	,,	,,	,,		,, B_1	
104.	2267	Animal Feed	Sandynalla, Ooty	23-3-79	,,	,, B_1 and B_2	Sheep Breeding Research Station
105.	2309	Groundnut Oilcake	Thadagam, Coimbatore	28-4-79	Good	,, B_1 and B_2	
106.	2316	Scented Supari Packet	,,	,,	,,	,, B_1 and B_2	
107.	2344	Dried Dates	Kallakkurichi	5-5-79	Insect damaged & whitish	,, B_1	
108.	2378	Groundnut Oilcake	,,	,,	Good	,, B_1	
109.	2318	Scented Supari Packet	Nattarmangalam	3-5-79	Good	Patulin	
110.	1965	Apple	Coimbatore	30-11-78	Rotten & Green mould on the rot	Penicillic acid	
111.	1428	Cotton Seed	Ooty	24-3-78	Good	Sterigmatocystin	
112.	1429	Cotton Seed	Ooty	24-3-78	,,	Sterigmatocystin	

Table No. 11 continued

Sl. No.	Name of the sample	Sample number and number of *Aspergillus niger*	*Aspergillus flavus*	*Aspergillus sp.*	*Penicillium sp.*	*Fusarium sp.*	*Mucor* sp.
1	2	3	4	5	6	7	8
2.	Wheat	18 (1.6); 20 (2.0); 22 (1.6); 42 (0.3); 44 (0.3); 47 (3.6); 171 (1.04); 175 (0.35); 180 (0.35); 188 (0.34); 190 (0.34); 210 (0.70); 213 (0.34); 214 (0.35); 217 (0.35); 219 (0.34); 303 (0.67); 335 (2.28); 412 (0.36); 489 (0.35); 494 (0.34); 507 (0.33); 532 (0.33); 533 (10.16); 569 (0.36); 680 (0.4); 682 (1.33); 704 (3.61); 711 (2.87); 770 (11.9); 778 (5.2); 781 (4.2); 785 (11.8); 790 (11.); 799 (10.6); 803 (3.7); 804 (0.75); 807 (1.2); 810 (0.69); 813 (0.12); 814 (0.35); 816 (1.21); 817 (1.45); 819 (0.35); 820 (0.36); 824 (2.5); 826 (1.30); 828 (0.35); 830 (0.34); 832 (0.34); 834 (1.77); 835 (0.35); 839 (0.35); 847 (3.3); 862 (1.22); 912 (1.15)	20 (2.6); 22 (3.3); 185 (0.37); 214 (0.35); 259 (1.25); 356 (0.36); 384 (0.69); 386 (0.69); 438 (0.34); 484 (0.67); 486 (1.21); 488 (1.76); 491 (0.34); 495 (0.34); 499 (0.35); 503 (1.15); 508 (0.35); 511 (1.56); 512 (0.33); 514 (0.33); 515 (0.66); 519 (3.3); 526 (0.33); 527 (3.3); 531 (4.12); 543 (0.80); 547 (1.52); 549 (4.45); 556 (26.2); 566 (0.72); 579 (4.82); 659 (1.31); 671 (1.26); 673 (0.68); 676 (0.68); 680 (0.4);	19 (1.6); 20 (2.6); 21 (0.6); 41 (0.6); 46 (0.6); 123 (2.99); 127 (0.32); 128 (1.8); 131 (0.69); 133 (0.36); 134 (2.86); 169 (0.69); 173 (0.38); 174 (1.5); 176 (0.35); 177 (0.36); 179 (0.65); 181 (7.12); 189 (0.7); 203 (0.6); 205 (0.35); 209 (2.46); 210 (1.05); 216 (1.1); 218 (3.8); 220 (0.70); 256 (0.35); 257 (0.72); 262 (0.35); 271 (0.34); 308 (3.4); 311 (0.37); 316 (0.35); 317 (0.36); 323 (9.96); 336 (3.38);	19 (0.3); 20 (0.3); 21 (0.3); 45 (0.6)	481 (0.35)	19 (5.6); 20 (5.3); 215 (0.36) 217 (1.16) 313 (1.85) 318 (1.85) 325 (0.69) 335 (0.36) 343 (3.5); 347 (1.24) 355 (2.3); 364 (2.95) 381 (0.35) 382 (0.68) 386 (0.35) 389 (1.84) 479 (2.32) 572 (0.36) 773 (4.2); 777 (6.8); 794 (4.4)

of colonies $\times 10^3$ (in parenthesis) /g of the sample

Rhizopus	*Trichoderma*	*Helmintho-sporium sp.*	Unidentified	Remarks (others)
9	10	11	12	13
22 (2.6); 129 (0.7);	215 (1.1);	188 (3.4);	71A (5.3); 72 (3.3); 97 (0.5);	*A. Unguis*
175 (6.9); 209 (0.37);	637 (6.15);	326 (0.68);	99 (4.3); 106 (0.6); 125 (5.5);	18 (0.3);
267 (0.33); 335 (1.2);		359 (0.35);	126 (0.37); 167 (0.36); 168 (0.36);	*Curvularia*
437 (1.52); 439 (0.36);		631 (2.92);	170 (0.36); 179 (9.74); 181 (0.35);	310 (0.36);
484 (1.12); 486 (2.53);		633 (3.15)	183 (0.33); 187 (0.36); 189 (1.84);	545 (0.70);
487 (0.34); 488 (3.96);			203 (0.72); 204 (0.38); 206 (0.37);	*Oospora*
489 (0.35); 492 (1.82);			208 (0.37); 210 (0.35); 215 (0.36);	317 (1.2);
468 (2.25); 504 (0.70);			221 (0.66); 222 (0.70); 223 (0.34);	327 (0.68);
509 (5.83); 510 (10.8);			224 (1.32); 225 (0.66); 226 (0.34);	*A. flaviceps*
512 (3.3); 513 (1.3);			227 (0.35); 258 (0.35); 260 (0.34);	507 (0.33)
514 (5.45); 516 (6.72);			264 (0.34); 265 (0.33); 272 (0.34);	
518 (2.4); 519 (5.83);			302 (2.28); 308 (0.34); 309 (0.34);	
527 (2.53); 531 (2.91);			312 (1.97); 328 (0.36); 329 (1.02);	
532 (2.86); 533 (1.12);			339 (0.74); 346 (0.35); 362 (0.37);	
535 (3.14); 537 (6.99);			365 (1.52); 368 (0.69); 374 (1.75);	
540 (3.82); 541 (0.36);			377 (4.21); 379 (3.29); 381 (0.70);	
544 (3.21); 547 (3.25);			412 (0.36); 428 (0.72); 432 (0.35);	
548 (2.12); 549 (8.15);			434 (0.34); 438 (1.81); 440 (1.02);	
550 (9.52); 552 (5.82);			443 (0.36); 479 (0.35); 480 (0.70);	
555 (0.69); 557 (1.25);			486 (3.3); 489 (0.35); 496 (0.34);	
559 (1.25); 566 (0.72);			500 (11.20); 502 (0.70); 503 (0.34);	
568 (4.82); 569 (1.56);			505 (0.35); 506 (0.72); 507 (0.33);	
573 (5.12); 575 (5.34);			511 (5.6); 515 (0.66); 527 (2.53);	
576 (5.02); 580 (1.23);			540 (0.36); 541 (0.36); 545 (0.35);	
854 (2.64); 535 (2.12);			555 (0.35); 563 (3.69); 564 (10.21);	
589 (4.98); 592 (2.51);			567 (1.65); 568 (7.87); 579 (5.99);	
593 (5.12); 593 (2.64);			582 (0.36); 588 (1.21); 596 (4.82);	
607 (2.99); 624 (1.62);			598 (6.54); 599 (4.52); 601 (6.66);	
640 (3.99); 656 (1.92);			604 (7.92); 609 (4.34); 626 (3.93);	
666 (1.69); 677 (1.62);			627 (3.60); 632 (6.01); 633 (2.89);	
679 (0.63); 680 (1.33);			635 (6.82); 638 (6.25); 639 (8.52);	
681 (0.77); 683 (2.66);			641 (17.74); 663 (0.38); 668 (0.66);	
692 (1.75); 694 (2.66);			669 (0.35); 670 (0.35); 672 (0.35);	
696 (1.53); 700 (4.23);			675 (0.67); 680 (0.4); 705 (0.38);	
701 (2.77); 706 (1.28);			706 (0.38); 710 (1.51); 711 (12.28);	
707 (0.8); 769 (7.9);			802 (30.6); 806 (0.37); 807 (1.5);	

Table No. 11 continued

Sl. No.	Name of the sample	Sample number and number of *Aspergillus niger*	*Aspergillus flavus*	*Aspergillus sp.*	*Penicillium sp.*
1	2	3	4	5	6
3.	Sorghum	48 (1.3); 57 (0.6); 58 (0.6); 59 (0.3)	-	-	-
4.	Bajra	17 (1.3)	-	-	17 (0.6)
5.	Samai	-	-	-	-
6.	Milo	23 (0.3)	-	-	-
7.	Maize	-	64 (0.3)	64 (0.9)	-
8.	Blackgram	62 (0.6)	-	-	62 (0.3)
9.	Greengram	932 (3.3)	-	-	-
10.	Redgram	851 (2.65); 934 (0.3)	934 (0.3)	-	-
11.	Beans	935 (0.6); 938 (2.6)	-	-	-
12.	Cowgram	-	-	-	-
13.	Rava	24 (2.3); 26 (6.0)	-	78 (0.6)	-
14.	Maida	25 (0.3); 49 (28.3); 50 (2.3)	25 (1.6)	-	-
15.	Groundnut oilcake	939 (0.6); 940 (2.3); 942 (4.0); 946 (3.3); 956 (3.3); 967 (1.6); 977 (0.3); 979 (1.0)	939 (0.6); 940 (1.3); 945 (26.0); 976 (0.6); 979 (0.3); 980 (0.6)	-	-
16.	Coconut oilcake	962 (2.3); 964 (1.3); 965 (1.3); 974 (0.3); 975 (2.0); 978 (0.3); 983 (0.3); 984 (3.6); 986 (0.3); 987 (2.6); 993 (1.3); 997 (1.0)	687 (0.4); 973 (3.6); 989 (0.3); 990 (4.3); 997 (1.3)	-	-
17.	Gingelly oilcake	966 (2.0)	-	-	-
18.	Paddy Bran	943 (0.3); 948 (0.3); 949 (1.3); 951 (0.3); 953 (3.0); 954 (2.0)	943 (0.6); 950 (3.0); 951 (0.6); 952 (6.3)	-	-
19.	Cattlefeed	971 (2.0)	970 (0.3)	-	-
20.	Poultry feed	1000 (0.6); 1003 (0.6)	1000 (13.0); 1001 (141.6); 1002 (75.3); 1005 (126.6); 1006 (14.3); 1008 (47.6); 1009 (26.3); 1012 (28.3); 1013 (22.3); 1014 (40.0); 1016 (38.3)	-	-

colonies $\times 10^3$ (in parenthesis) /g of the sample						
Fusarium sp.	*Mucor*	*Rhizopus*	*Trichoderma*	*Helmintho-sporium sp.*	Unidentified	Remarks (others)
7	8	9	10	11	12	13
-	27 (2.3)	-	-	-	100 (0.9); 620 (4.24)	-
-	-	-	-	-	-	-
-	-	-	-	-	60 (7.8); 61 (34.8)	*Cladosporium* 60 (2.3)
-	-	-	-	-	-	-
-	-	-	-	-	-	-
-	-	933 (1.6)	-	-	108 (4.9); 371 (12.0); 933 (2.6)	-
-	-	-	-	-	-	-
-	937 (3.3)	852 (1.11)	-	-	852 (1.77); 853 (5.2); 934 (0.3)	-
-	-	935 (1.6)	-	-	-	-
-	-	936 (1.0)	-	-	-	-
-	80 (0.6)	24 (4.0); 80 (1.6)	-	-	24 (2.0); 86 (3.9); 103 (0.3)	-
-	25 (0.3)	-	-	-	79 (0.9); 81 (2.2); 101 (0.3); 102 (0.3); 110 (0.1)	-
-	955 (3.6); 994 (1.6)	946 (1.3); 957 (2.0); 995 (1.3); 996 (1.3)	-	-	940 (1.6); 941 (22.6); 976 (0.6); 979 (0.6)	-
-	959 (2.0); 992 (2.0)	960 (3.0); 981 (3.6); 983 (0.3); 988 (1.3); 991 (1.6); 997 (0.6)	989 (11.6);	-	958 (0.3); 960 (0.6); 961 (0.3); 974 (0.3); 978 (0.9); 985 (0.9); 986 (0.6); 997 (0.3)	-
-	-	-	-	-	-	-
-	-	947 (3.0); 951 (0.3); 952 (1.3)	-	-	954 (5.3)	-
-	-	968 (1.6); 969 (2.3); 970 (2.0)	-	-	968 (0.3)	-
-	-	999 (4.6); 1000 (4.6); 1003 (0.6); 1010 (5.3); 1011 (5.0); 1015 (3.3)	-	-	998 (12.6); 999 (5.3); 1000(0.6); 1002 (9.6); 1003 (2.6); 1004 (19.3); 1006 (15.6); 1007 (33.0); 1010 (8.6); 1011 (7.3); 1015 (9.0)	-

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Appendix I

Mould in Eatables is a Health Hazard*

By Dr. T. Balasubramanian and
Dr. S. Neelakantan

Groundnut, the main cash crop of South Arcot District, is harvested in winter in pouring rains. The peasants try their best to keep the soaked groundnuts in airy verandahs in their houses and whenever there is sunshine, one sees nothing but groundnuts spread on the roads everywhere for drying. But within this short period of storage, the nuts begin to turn mouldy.

This mould development lowers the quality of the groundnut and also poses a serious health hazard for the consumers. The high humidity in the air due to the rainy atmosphere and the relatively high moisture content of the freshly harvested groundnuts are very conducive for the growth of fungi on them. The weather continuing to be bad, heavy mould growth with the consequent production of toxic substances called mycotoxins will occur. This situation can be avoided, if proper drying facilities with necessary machinery are made available in the villages.

Not only groundnut, but almost all the food crops are susceptible to mould attack during their entire post-harvest periods of storage and marketing, due to their initial relatively high moisture content. Moulds do grow on fruits, vegetables, nuts and food products like jam, pickles, papads etc. or on any food you name. It is a common practice among the people to remove the mould growth and consume the food or it is diverted to feed the animals. Even though the outer mouldy growth is removed, the mycotoxins that might have been secreted by them into the food cannot be removed. The mycotoxins affect the vital organs like liver and kidney and some of them are carcinogenic (encouraging cancer) too. Hence, the presence of moulds in foods or animal feeds should be viewed with serious concern. The mycotoxins, once they enter the animal body, will get into the animal products viz., milk, meat and eggs thereby endangering human health.

There are many mycotoxins like aflatoxin, penicillic acid, patulin, sterigmatocystin, ochratoxin, citrinin, zearalenone — to name a few — produced by the fungi of the genera *Aspergillus, Penicillium* and *Fusarium.* The only possible way of avoiding contamination of the foods by mycotoxins is to protect them from the invasion and growth of fungi on them. For that, proper drying of the crops, soon after harvest, is necessary coupled with safe storage practices. The storage should be done in properly ventilated space and storage structures.

Of course, in the case of horticultural produce like fruits and vegetables, correct post-harvest handling is required. These are perishable goods liable for rapid spoilage. Proper washing of the crop with good water to remove the adhering soil, dust etc. is necessary. Careful handling to avoid physical damage, proper packing and prompt cooling will greatly reduce spoilage. Damaged, bruised and rotten fruits and vegetables should be separated from

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the good and healthy ones after harvesting, during transportation and during marketing. These procedures will minimise mould contamination.

To get to the mouldy groundnuts. These are used to extract oil in local mills. It is now well-known that a species of moulds belonging to the *Aspergillus* genus grows profusely on groundnuts not only spoiling them but also secreting enormous amounts of a mycotoxin known as aflatoxin. This mycotoxin present in them definitely gets into the extracted oil and the pressed oilcake. Only during the refining process of the oil, the mycotoxin in the oil gets destroyed. However in villages people use mostly the unrefined oil only. In foreign countries, ultraviolet lamps are employed coupled with electronic devices to pick out mouldy groundnuts, before processing them. In our country, such advanced technology is not possible at present and consequently, a small proportion of the mycotoxin goes into the oil and the greater fraction of it is retained in cake itself. This oilcake, when fed to the cattle or poultry causes production of aflatoxin-contaminated milk, eggs etc.

Besides, many food items, like groundnut candies etc. are also prepared using groundnuts and sold in the markets. Here again, no pains are taken by the producers to separate the spoiled kernels. As a result, the mycotoxin gets into the human system. Likewise, wherever we turn, the same problems of mould faces us. Especially for the poor people, who are already malnourished, the intake of mycotoxin-contaminated foods over a period of years poses serious health problem.

Thus, it becomes obvious that much attention has to be diverted in this direction by the Government agencies, processors and consumers alike. An awareness to the seriousness of the problem has to be created and as far as possible, efforts should be made to prevent fungal growth on foods.

Appendix II

Hidden Enemy in Stored Foods and Feeds★

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India is an agriculture based country and its food production had been steadily increasing. India would be self-sufficient in foodgrains, but for the huge avoidable losses during storage, processing and marketing. It has been estimated that about 50% of our grains are retained by the farmers, and the remaining finds its way to the various warehousing agencies and traders before they reach their point of consumption. Scientific principles of storing grains are unknown to farmers. The rural traditional storage structures and practices are in vogue since generations without much modifications. These are far below the standards that have been set forth by the application of modern research. Even the various warehousing and procuremnt agencies like Food Corporation of India, Central Warehousing Corporation, State Warehousing Corporations and Civil Supply Corporations etc. are unable to store the grains in the ideal conditions that are least conducive to any damage.

Adequate protected storage space is not available in our country. The acuteness of the problem was felt in all its severity recently, when our government agencies were forced to use even abandoned airstrips and other open fields to store millions of tonnes of foodgrains When scientific methods of storage are not practised, there are both quantitative and qualitative losses that occur in stored products. The quantitative losses are mainly caused by rodents, insects and micro-organisms. Due to excessive heating and moisture, the food suffers qualitatively in the development of discolouration, of smell, loss of nutrients etc:

The warehousing staff have so far been placing emphasis mainly on control of rodents and insects. Since the quantitative loss due to microbial damage is comparatively less, enough attention has not been focused upon them. However the grains suffer severely qualitywise due to micro-organisms and recent research has shown that the fungal growth on foods results in the production of various toxins. In this article, we have made an attempt to highlight the storage problems caused by fungi, mycotoxins and their effect on human being and possible ways of prevention or control of this damage.

Fungal spores are present everywhere and they multiply and spread fast on the foods when conditions are favourable to their growth. All they usally need to start their process of multiplication on any food is a little moisture. There are hundreds of varieties of fungi. Though many are not toxic, some are supertoxic. When they grow, they secrete products of their metabolism. Some of these toxic fungal metabolites are called mycotoxins. These mycotoxins have been demonstrated to be toxic to both animals and human beings alike, and they can cause various diseases including cancer. The diseases

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caused by the mycotoxins are termed mycotoxicoses. Liver and kidney are the mainly affected parts in mycotoxicoses.

Though it has been known for many years that moulds produce toxic metabolites, their, effects were largely ignored. Thus mycotoxicoses have been aptly called 'the neglected diseases'. This situation has altered drastically with the developments relative to "Turkey X" disease which appeared in England in 1960. Millions of young turkey poults died because their feed was contaminated by the mould, *A. flavus*. Later, it was found that the diet contained aflatoxin which is an extremely potent carcinogen. Aflatoxin is produced by the fungus *A. flavus*. Because of its significance in problems of animal and human health worldwide involvement focused intense attention upon other mycotoxins too. There are many other mycotoxins namely sterigmatocystin, patulin, penicillic acid, ochratoxin, citrinin, rubratoxin, diacetoxyscirpenol etc., which are also equally harmful to both human beings and animals. The presence of mould toxins is potentially the most serious quality problem which faces consumers, storage agencies, manufacturers and handlers of food and feed products. So, an awareness of the problem of mycotoxin is most important and urgently required especially in India. Here, in our country when fungal growth is observed on any food, say for example, pickle or papad, the fungus is removed from the top manually and the food is consumed. Even when the particular foodstuff becomes totally inedible, both tastewise and odourwise, it is diverted for feeding the animals, without realising the potential health hazard that might arise out of it. This practice should be abolished, and it can be done only by creating an awareness of the effects of the mycotoxin intake among the people.

In the interest of public health suitable methods should be adopted to minimise mycotoxin contamination. Since prevention is better than cure, one should control or stop the growth of of fungi on the stored materials. The growth of fungi on stored commodities is influenced by the moisture content of the food and feeds and the relative humidity of its environment. Hence fungal spoilage in foods and feeds can be primarily controlled by drying them to a safe moisture level of 7–13% depending upon the commodity. Each species of fungi requires an optimum humidity for maximum growth and generally speaking, high humidity favours rapid growth of fungi. Besides relative humidity, the length of storage period, type of foodgrains stored, temperature of storage and the peculiarities of the fungus etc., also play a role in the growth of fungi and production of toxins. Lowering the storage temperature to below 5°C reduces the proliferation of most of the fungi. Infestation by insect pests in stored foods and feeds also increases the moisture content. When insects grow, they release enough moisture by their respiration to promote the growth of fungi. Once the invasion of fungi begins, their multiplication is self-accelerating and leads to progressive deterioration.

Groundnut is highly susceptible for attack by the fungus, *A flavus*. Storage of groundnuts in jute bags as against in metal containers were found to be less conductive to aflatoxin formation, possibly due to better aeration in bags mitigating the accumulation of moisture. The same may hold good for grains too. Pockets of grains of high moisture content than the surrounding bulk, especially inside an enclosed bin can cause severe deterioration. In such cases, localized growth of the storage fungi occurs first and they eventually spread throughout the stored material. In big godowns, where they stack hundreds and thousands of tonnes of foodgrains, proper ventilation should be provided to dissipate the heat. This in turn minimises the fungal growth. Again fumigants are also being used in godowns to minimise the insect infestation. Use of fumigants reduces the growth of storage fungi directly by being fungicides, and indirectly reducing the moisture released by the insects. These moulds-producing mycotoxins are not the monopoly products of the godowns storage alone, but they are proliferating even in the domestic storage of bottles and pots and among the mycotoxins, aflatoxin is commonly seen everywhere.

From groundnuts, the aflatoxin gets into the human system in more than one way. During oil extraction, the infected seeds are not removed from good kernels, and as a result aflatoxin gets into groundnut oil too. It is not completely destroyed even by heating the oil to its smoking point. But it gets destroyed during the refining process. Hence, whenever possible, use of refined oil is advisable to avoid aflatoxin entering the human system through the oil. It has been reported that aflatoxin M has been found in the milk of animals eating feeds contaminated with aflatoxin in several samples of milk in South Africa. So by feeding the cattle with aflatoxin containing oilcakes, the toxin gets into the human system indirectly. In the case of poultry, intake of feeds containing aflatoxin results in their high mortality rate and lower egg production. However, aflatoxin has also been found to get into the egg.

In a recent survey, at the Tamil Nadu Agricultural University it has been found that sterigmatocystin, a carcinogenic toxin was present in twenty samples out of 1000 samples of foodgrains collected from different parts of Tamil Nadu. Similarly, zearalenone, a carcinogenic toxin was detected in a majority of the samples collected from grains stacked and stored on open airstrips and grounds.

It has been reported that malnutrition and mycotoxins coexist in many areas of the world where there is high incidence of liver diseases. It is also true that only poorly nourished people who have least protective mechanisms against the toxic effects of mycotoxins, consume such damaged foods for they are available at lesser costs So, it is highly essential that an awareness to the fungal toxins in food should be created amongst our people and the warehousing staff who are involved in the public foodgrains distribution system.

In conclusion, the following suggestions may be followed to minimize the contamination of stored products by the inbred enemy, the mycotoxin and the causative fungi.

1. Proper agronomic practices may be developed to grow different varieties that are resistant to fungal attack.

2. Proper harvest and post harvest practices may be followed so as to reduce fungal contamination.

3. The materials to be stored should be dried to a safe moisture level, before storage.

4. The commodities should be stored in protected space with adequate ventilation facilities.

5. By proper application of fumigants, growth of fungi and insects should be controlled.

6. Mouldy foods and feeds should be rejected as far as practicable.

7. The stored foods and feeds should be dried periodically, under the sun.